Food and Drug Administration, HHS

Food and Drug Administration. The raw data and results from each potency test performed shall be included.

(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 E—Bacillus of Calmette and Guerin (BCG) Vaccine

§ 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 $-20\,^\circ\text{C}$ or colder, or in a frozen state at $-70\,^\circ\text{C}$ 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 injection not more than 10 U.S. Tuberculin Units, Purified Protein Derivative, 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...
§ 620.42 Production.

(a) BCG inoculum. The inoculum of BCG used for seed lot or production of final lot in seed buildup must have been removed from the preceding seed lot in accordance with the following passage and time schedule:

(1) No more than 3 passages from primary to secondary seed lot within a 2-month period.

(2) If no secondary seed lot is used, no more than 9 passages from primary seed lot to final lot within a 6-month period.

(3) No more than 9 passages from secondary seed lot to final lot within a 6-month period.

(b) Propagation of bacteria. The culture medium for propagation of BCG Vaccine must not contain ingredients known to be capable of producing allergic effects in humans or of causing the bacteria to become virulent for guinea pigs. The growth in each container must be examined visually, and only those cultures that have the typical growth pattern characteristic of BCG are to be used in a vaccine.

(c) Colony-forming units (CFU) before and after freeze-drying. Each lot of BCG Vaccine must be tested to determine the number of CFU per individual final container both before and after freeze-drying. The upper and lower limits of the viable count are to be established by the manufacturer of the vaccine for the particular route of administration recommended and must be specified in the license application. The loss in viability after drying must not exceed 90 percent.

§ 620.42 Production.

(a) BCG inoculum. The inoculum of BCG used for seed lot or production of final lot in seed buildup must have been removed from the preceding seed lot in accordance with the following passage and time schedule:

(1) No more than 3 passages from primary to secondary seed lot within a 2-month period.

(2) If no secondary seed lot is used, no more than 9 passages from primary seed lot to final lot within a 6-month period.

(3) No more than 9 passages from secondary seed lot to final lot within a 6-month period.

(b) Propagation of bacteria. The culture medium for propagation of BCG Vaccine must not contain ingredients known to be capable of producing allergic effects in humans or of causing the bacteria to become virulent for guinea pigs. The growth in each container must be examined visually, and only those cultures that have the typical growth pattern characteristic of BCG are to be used in a vaccine.

(c) Colony-forming units (CFU) before and after freeze-drying. Each lot of BCG Vaccine must be tested to determine the number of CFU per individual final container both before and after freeze-drying. The upper and lower limits of the viable count are to be established by the manufacturer of the vaccine for the particular route of administration recommended and must be specified in the license application. The loss in viability after drying must not exceed 90 percent.

§ 620.42 Production.

(a) BCG inoculum. The inoculum of BCG used for seed lot or production of final lot in seed buildup must have been removed from the preceding seed lot in accordance with the following passage and time schedule:

(1) No more than 3 passages from primary to secondary seed lot within a 2-month period.

(2) If no secondary seed lot is used, no more than 9 passages from primary seed lot to final lot within a 6-month period.

(3) No more than 9 passages from secondary seed lot to final lot within a 6-month period.

(b) Propagation of bacteria. The culture medium for propagation of BCG Vaccine must not contain ingredients known to be capable of producing allergic effects in humans or of causing the bacteria to become virulent for guinea pigs. The growth in each container must be examined visually, and only those cultures that have the typical growth pattern characteristic of BCG are to be used in a vaccine.

(c) Colony-forming units (CFU) before and after freeze-drying. Each lot of BCG Vaccine must be tested to determine the number of CFU per individual final container both before and after freeze-drying. The upper and lower limits of the viable count are to be established by the manufacturer of the vaccine for the particular route of administration recommended and must be specified in the license application. The loss in viability after drying must not exceed 90 percent.
§ 620.43 Reference BCG Vaccine.

A reference BCG Vaccine, for use in determining the validity of the test for colony-forming units, is to be obtained from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.


§ 620.44 Potency tests.

(a) Colony-forming units (CFU). The number of CFU must be determined on the contents of each of at least 10 individual final containers of each lot of BCG Vaccine. Of the 10 or more individual final containers, the contents of at least 5 before, and an equal number after, freeze-drying must be tested. Final containers of the freeze-dried vaccine are to be reconstituted as for human use with the diluent recommended by the manufacturer. The number of CFU to be reported for each lot of BCG Vaccine must be determined only from test tubes containing between 10 and 50 CFU. Dilutions must be made as follows:

1. Dilutions are made from an appropriate volume of the liquid vaccine before freeze-drying or the reconstituted vaccine after freeze-drying. Appropriate dilutions are made with modified Youman's medium specified in paragraph (a)(4) of this section, up to a point where subsequent serial half-log dilutions will result in at least 1 tube containing between 10 and 50 CFU.

2. Serial half-log dilutions are made in 16×125 millimeter screw-capped test tubes into which 4.5 milliliter aliquots of the diluent prescribed in paragraph (a)(4) of this section have been dispensed. Two milliliters of thoroughly mixed vaccine are added to the first tube of the half-log series, mixed thoroughly, and 2.0 milliliters from this tube are transferred to the next tube in the series. The process of mixing and serially transferring 2.0 milliliters is repeated through each consecutive tube and 2.0 milliliters are discarded from the last tube.

3. After the serial half-log dilutions are completed, 0.5 milliliter of 1.5 percent agar solution that has been cooled to 42° C is quickly added, where necessary, to make a final concentration of 0.15 percent agar, and the contents of the tubes are thoroughly mixed. After mixing, all tubes are incubated at 35° to 37° C for 3 to 4 weeks.

4. The composition of modified Youman's medium with bovine albumin is as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>5.0 grams</td>
</tr>
<tr>
<td>Monopotassium phosphate (KH₂PO₄)</td>
<td></td>
</tr>
<tr>
<td>Potassium sulfate (K₂SO₄)</td>
<td>0.5 grams</td>
</tr>
<tr>
<td>Magnesium citrate</td>
<td>1.5 grams</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>19.0 grams</td>
</tr>
<tr>
<td>Glycerine</td>
<td>20.0 milliliters</td>
</tr>
<tr>
<td>Distilled water q.s. to</td>
<td>900.0 milliliters</td>
</tr>
</tbody>
</table>
| One hundred milliliters of 5-percent aqueous solution of bovine albumin that has been sterilized by filtration are added to the Youman's medium to produce a final concentration of 0.5 percent of bovine albumin. The pH is adjusted to 7.0 with 5N sodium hydroxide.

(b) Intradermal guinea pig test. Two or more guinea pigs, each weighing no less than 250 grams, must be injected intradermally in 4 different sites with the following amounts and dilutions of each lot of BCG Vaccine:

1. Vaccine intended for intradermal injection is reconstituted as for human use with the diluent recommended by the manufacturer. One-tenth milliliter of reconstituted vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) of the reconstituted vaccine are injected into the guinea pigs. The diluent for the ten-fold dilutions is isotonic solution for injection.

2. Vaccine intended for percutaneous injection into humans is reconstituted with the diluent recommended by the manufacturer so that at least one human dose (estimated to be within a range of from 1 to 3×10⁵ CFU) is contained in 0.1 milliliter. A narrower range of CFU is determined for each specific vaccine by the manufacturer and specified in the license application. One-tenth milliliter of the selected dose of vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) are injected into the guinea pigs. The diluent for the ten-fold dilutions is an isotonic solution for injection.

3. The lot of vaccine is satisfactory if:
§ 620.45 Test for freedom from virulent mycobacteria.

(a) Each lot of BCG Vaccine must be tested to determine that it does not contain virulent mycobacteria. The test must be performed using at least 6 guinea pigs, each weighing between 250 and 300 grams. Vaccine intended for intradermal injection in humans must be tested by injecting into guinea pigs the number of bacteria contained in at least 50 human doses. Vaccine intended for percutaneous use in humans must be tested by injecting into guinea pigs 50 times the number of bacteria estimated to be introduced parenterally into humans by the recommended procedure. The vaccine for all tests must be inoculated subcutaneously or intramuscularly into the guinea pigs. All animals that die during the observation period must be examined post-mortem. All animals that survive the observation period must be sacrificed and examined post mortem. The lot passes the test if at least two-thirds of the animals on test survive an observation period of not less than 6 weeks, and if the post-mortem examination reveals no evidence of tuberculosis in any of the test animals.

(b) If any virulent mycobacteria are found in any lot of BCG Vaccine, whether or not the manufacturer intends to submit samples and protocols of this lot to the Center for Biologics Evaluation and Research for release, the following actions must be taken:

(1) In addition to the requirements of §§600.12 and 600.14 of this chapter, the manufacturer shall immediately report by telephone, telegraph, or cable the finding of virulent mycobacteria to the Director, Center for Biologics Evaluation and Research.

(2) All production and distribution of lots of BCG Vaccine produced from the same secondary seed lot as the contaminated lot of BCG Vaccine must be discontinued. If no secondary seed lot is used the same requirements apply to the primary seed lot.

(3) The manufacturer shall conduct a thorough and prompt investigation concerning the failure of the lot to meet the required safety and purity specifications, including retesting the suspect lot and the source secondary seed lot (or primary seed lot, if no secondary seed lot is used) and shall undertake a thorough review of all manufacturing records and procedures to determine the probable cause of the failure.

(4) A written record of the investigation, including the retest results, must be submitted to the Director, Center for Biologics Evaluation and Research.

(5) Neither production nor distribution of BCG Vaccine may be resumed until the manufacturer is notified in writing by the Director, Center for Biologics Evaluation and Research, that such activity may be resumed.

§ 620.46 General requirements.
(a) Dose. These standards are based on (1) vaccine intended for intradermal injection in a single human immunizing dose of 0.1 milliliter and (2) vaccine intended for percutaneous injection in a single skin application through which inoculation is made by a multiple puncture device.
(b) Date of manufacture. The date of manufacture is the date of initiation of the last valid determination for CFU after freeze-drying.

§ 620.47 Labeling.
In addition to conforming to the applicable requirements of §§ 610.60, 610.61, and 610.62 of this chapter, the package label must bear the following information:
(a) Specification of the route of administration.
(b) A statement that the vaccine contains live bacteria and should be protected against exposure to light.
(c) A statement that the vaccine must be administered within 8 hours after reconstitution, and that reconstituted vaccine not used within 8 hours must be discarded.

§ 620.48 Samples; protocols; official release.
(a) For each lot of vaccine, the following materials must be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.
(1) Samples and diluent that will provide at least 20 milliliters when the samples are reconstituted as recommended in the package insert by the manufacturer of the vaccine.
(2) A protocol that consists of a complete summary of the manufacture of each lot, including all results of each test required by all applicable regulations. If the protocol is not included in the shipment of the samples, it must be sent promptly to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.
(b) The BCG Vaccine must not be issued by the manufacturer until written notification of official release is received from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration.