**FINAL BULK VACCINE**
The final bulk vaccine is prepared from a single harvest or by pooling a number of single harvests. A stabiliser may be added; if the stabiliser interferes with the determination of bacterial concentration in the final bulk vaccine, the determination is carried out before addition of the stabiliser.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

- **Bacterial and fungal contamination.** Carry out the test for sterility (2.6.1), using 10 ml for each medium. The final bulk vaccine complies with the test for sterility except for the presence of mycobacteria.

- **Count of viable units.** Determine the number of viable units per millilitre by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable biochemical method. Carry out the test in parallel on a reference preparation of the same strain.

- **Bacterial concentration.** Determine the total bacterial concentration by a suitable method, either directly by determining the mass of the micro-organisms, or indirectly by an opacity method that has been calibrated in relation to the mass of the organisms; if the bacterial concentration is determined before addition of a stabiliser, the concentration in the final bulk vaccine is established by calculation. The total bacterial concentration is within the limits approved for the particular product.

- The ratio of the count of viable units to the total bacterial concentration is not less than that approved for the particular product.

**FINAL LOT**
The final bulk vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine; the containers are closed either under vacuum or under an inert gas.

Except where the filled and closed containers are stored at a temperature of ~20 °C or lower, the expiry date is not later than 4 years from the date of harvest.

Only a final lot that complies with the following requirement for count of viable units with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot. Provided the test for excessive dermal reactivity has been carried out with satisfactory results on the working seed lot and on 5 consecutive final lots produced from it, the test may be omitted on the final lot.

**Count of viable units.** Determine the number of viable units per millilitre of the reconstituted vaccine by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable biochemical method. The ratio of the count of viable units after freeze-drying to that before is not less than that approved for the particular product.

**TESTS**

- **Virulent mycobacteria.** Inject subcutaneously or intramuscularly into each of 6 guinea-pigs, each weighing 250-400 g and having received no treatment likely to interfere with the test, a quantity of vaccine equivalent to at least 50 human doses. Observe the animals for at least 42 days. At the end of this period, kill the guinea-pigs and examine by autopsy for signs of infection with tuberculosis, ignoring any minor reactions at the site of injection. Animals that die during the observation period are also examined for signs of tuberculosis. The vaccine complies with the test if none of the guinea-pigs shows signs of tuberculosis and if not more than 1 animal dies during the observation period. If 2 animals die during this period and autopsy does not reveal signs of tuberculosis repeat the test on 6 other guinea-pigs. The vaccine complies with the test if not more than 1 animal dies during the 42 days following the injection and autopsy does not reveal any sign of tuberculosis.

- **Bacterial and fungal contamination.** The reconstituted vaccine complies with the test for sterility (2.6.1) except for the presence of mycobacteria.

- **Excessive dermal reactivity.** Use 6 healthy, white or pale-coloured guinea-pigs, each weighing not less than 250 g and having received no treatment likely to interfere with the test. Inject intradermally into each guinea-pig, according to a randomised plan, 0.1 ml of the reconstituted vaccine and of 2 tenfold serial dilutions of the vaccine and identical doses of the comparison vaccine. Observe the lesions formed at the site of the injection for 4 weeks. The vaccine complies with the test if the reaction it produces is not markedly different from that produced by the comparison vaccine.

- **Temperature stability.** Maintain samples of the freeze-dried vaccine at 37 °C for 4 weeks. Determine the number of viable units in the heated vaccine and in unheated vaccine as described below. The number of viable units in the heated vaccine is not less than 20 per cent that in unheated vaccine.

- **Water.** Not more than the limit approved for the particular product, determined by a suitable method.

**ASSAY**
Determine the number of viable units in the reconstituted vaccine by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable validated biochemical method. The number is within the range stated on the label. Determine the number of viable units in the comparison vaccine in parallel.

**LABELLING**
The label states:
- the minimum and maximum number of viable units per millilitre in the reconstituted vaccine,
- that the vaccine must be protected from direct sunlight.

**CHOLERA VACCINE**

**Vaccinum cholerae**

**DEFINITION**
Cholera vaccine is a homogeneous suspension of a suitable strain or strains of *Vibrio cholerae* containing not less than 8 × 10^7 bacteria in each human dose. The human dose does not exceed 1.0 ml.

---

**IDENTIFICATION**

BCG vaccine is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property and by the characteristic appearance of colonies grown on solid medium. Alternatively, molecular biology techniques (for example nucleic acid amplification) may be used.

---

**01/2005:0154**
PRODUCTION
The vaccine is prepared using a seed-lot system. The vaccine consists of a mixture of equal parts of vaccines prepared from smooth strains of the 2 main serological types, Inaba and Ogawa. These may be of the classical biotype with or without the El-Tor biotype. A single strain or several strains of each type may be included. All strains must contain, in addition to their type O antigens, the heat-stable O antigen common to Inaba and Ogawa. If more than one strain each of Inaba and Ogawa are used, these may be selected so as to contain other O antigens in addition. The World Health Organisation recommends new strains which may be used if necessary, in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. In order to comply with the requirements for vaccination certificates required for international travel, the vaccine must contain not less than $8 \times 10^9$ organisms of the classical biotype. Each strain is grown separately. The bacteria are inactivated either by heating the suspensions (for example, at 56 °C for 1 h) or by treatment with formaldehyde or phenol or by a combination of the physical and chemical methods.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9) modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION
It is identified by specific agglutination tests.

TESTS
Phenol (2.5.15). If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antibody production. Test the ability of the vaccine to induce antibodies (such as agglutinating, vibriocidal or haemagglutinating antibodies) in the guinea-pig, the rabbit or the mouse. Administer the vaccine to a group of at least 6 animals. At the end of the interval of time necessary for maximum antibody formation, determined in preliminary tests, collect sera from the animals and titrate them individually for the appropriate antibody using a suitable method. The vaccine to be examined passes the test if each serotype has elicited a significant antibody response.

Sterility (2.6.1). It complies with the test for sterility.

LABELLING
The label states:
– the method used to inactivate the bacteria,
– the number of bacteria in each human dose.

01/2005:0155

CHOLERA VACCINE, FREEZE-DRIED

Vaccinum cholerae cryodesiccatum

DEFINITION
Freeze-dried cholera vaccine is a preparation of a suitable strain or strains of *Vibrio cholerae*. The vaccine is reconstituted as stated on the label to give a uniform suspension containing not less than $8 \times 10^9$ bacteria in each human dose. The human dose does not exceed 1.0 ml of the reconstituted vaccine.

PRODUCTION
The vaccine is prepared using a seed-lot system. The vaccine consists of a mixture of equal parts of vaccines prepared from smooth strains of the 2 main serological types, Inaba and Ogawa. These may be of the classical biotype with or without the El-Tor biotype. A single strain or several strains of each type may be included. All strains must contain, in addition to their type O antigens, the heat-stable O antigen common to Inaba and Ogawa. If more than one strain each of Inaba and Ogawa are used, these may be selected so as to contain other O antigens in addition. The World Health Organisation recommends new strains which may be used if necessary, in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. In order to comply with the requirements for vaccination certificates required for international travel, the vaccine must contain not less than $8 \times 10^9$ organisms of the classical biotype. Each strain is grown separately. The bacteria are inactivated either by heating the suspensions (for example, at 56 °C for 1 h) or by treatment with formaldehyde or phenol or by a combination of the physical and chemical methods. Phenol is not used in the preparation. The vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine. The containers are then closed so as to exclude contamination.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9) modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION
The vaccine reconstituted as stated on the label is identified by specific agglutination tests.

TESTS
Phenol (2.5.15). If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antibody production. Test the ability of the vaccine to induce antibodies (such as agglutinating, vibriocidal or haemagglutinating antibodies) in the guinea-pig, the rabbit or the mouse. Administer the reconstituted vaccine to a group of at least 6 animals. At the end of the interval of time necessary for maximum antibody formation, determined in preliminary tests, collect sera from the animals and titrate them individually for the appropriate antibody using a suitable method. The vaccine to be examined passes the test if each serotype has elicited a significant antibody response.

Sterility (2.6.1). The reconstituted vaccine complies with the test for sterility.

LABELLING
The label states:
– the method used to inactivate the bacteria,
– the number of bacteria in each human dose.