Edition: BP 2025 (Ph. Eur. 11.6 update)

Clostridium Botulinum Vaccine

General Notices

Botulinum Vaccine

(Clostridium Botulinum Vaccine for Veterinary Use, Ph. Eur. monograph 0360)

When Clostridium Botulinum Vaccine or Botulinum Vaccine is prescribed or demanded and the types to be present are not stated, Clostridium Botulinum Vaccine prepared from types C and D shall be dispensed or supplied.

Ph Eur

1 DEFINITION

Clostridium botulinum Vaccine for veterinary use is prepared from liquid cultures of suitable strains of *Clostridium botulinum* type C or type D or a mixture of these types. The whole culture or its filtrate or a mixture of the two is inactivated to eliminate its toxicity while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for active immunisation of animals against botulism.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

C. botulinum Used for production is grown in an appropriate liquid medium.

The preparation may be adsorbed, precipitated or concentrated. It may be treated with a suitable adjuvant and may be freeze-dried.

2-2 CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the animals for which it is intended.

The following test for safety (section 2-2-1) may be used during the demonstration of safety.

2-2-1 Safety

Carry out the tests for each route and method of administration to be recommended for vaccination and where applicable, in animals of each category for which the vaccine is intended, using in each case animals not older than the minimum age to be recommended for vaccination. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test, use not fewer than 8 animals that do not have antibodies against *C. botulinum*. Administer to each animal 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer another dose after an interval of at least 14 days. Observe the animals at least daily until 14 days after the last administration.

The vaccine complies with the test if no animal shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-3 MANUFACTURER'S TESTS

2-3-1 Batch potency test

It is not necessary to carry out the potency test (section 3-4) for each batch of the vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method (e.g. a cell-based assay) is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

3 BATCH TESTS

The identification, the tests and the determination of potency apply to the liquid preparation and to the freeze-dried preparation reconstituted as stated on the label.

3-1 Identification

When injected into a healthy animal free from antibodies against the type or types of *C. botulinum* from which the vaccine was prepared, the vaccine stimulates the production of such antibodies.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Residual toxicity

Inject 0.5 mL of the vaccine by the subcutaneous route into each of 5 mice, each weighing 17-22 g. Observe the animals at least daily for 7 days.

The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine.

3-4 Potency

Use for the test healthy white mice from a uniform stock, each weighing 18-20 g. Use as challenge dose a quantity of a toxin of *C. botulinum* of the same type as that used in the preparation of the vaccine corresponding to 25 times the paralytic dose 50 per cent (a paralytic dose 50 per cent being the quantity of toxin that, when injected by the intraperitoneal route into mice, causes paralysis in 50 per cent of the animals within an observation period of 7 days). If 2 types of *C. botulinum* have been used in the preparation of the vaccine, carry out the potency determination for each. Dilute the vaccine to be examined 8-fold using a 9 g/L solution of *sodium chloride R*. Inject 0.2 mL of the dilution subcutaneously into each of 20 mice. After 21 days, inject the challenge dose by the intraperitoneal route into each of the vaccinated mice and into each of 10 control mice. Observe the mice for 7 days and record the number of animals that show signs of botulism.

The test is not valid unless all the control mice show signs of botulism during the observation period. The vaccine complies with the test if not fewer than 80 per cent of the vaccinated mice are protected.

Application of alternative end-points Once a laboratory has established the above assay for routine use, the lethal end-point is replaced by observation of clinical signs and application of an end-point earlier than death to reduce animal suffering. The following is given as an example.

The progress of botulinum infection in mice following intraperitoneal injection can be represented by 6 stages defined by typical clinical signs:

Stage 1: increased rate of breathing;
Stage 2: increased rate of breathing, slight hollowing of the flanks;
Stage 3: increased rate of breathing, noticeable hollowing of the flanks, slight orbital tightening of the eyes;

Stage 4: difficulty in breathing, noticeable hollowing of the flanks, pilo-erection, abnormal gait and partial loss of mobility, moderate orbital tightening of the eyes;
Stage 5: very laboured breathing, severe hollowing of the flanks, pilo-erection, mouse is immobile and unresponsive to external stimuli (moribund state), definite orbital tightening of the eyes;
Stage 6: death.

Mice are observed at least twice daily after challenge. Clinical signs are recorded using a chart. The end-point stage is defined as the earliest stage that yields assay results equivalent to those obtained when a lethal end-point is applied, as established during validation. Mice reaching the pre-defined end-point stage are removed and euthanised at specified observation times.

The application of alternative end-points must be verified by each laboratory by scoring a suitable number of assays using both the clinical signs and the lethal end-point.

4 LABELLING

The label states:

— the type or types of *C. botulinum* from which the vaccine has been prepared;

— whether the preparation is a toxoid or a vaccine prepared from a whole inactivated culture or a mixture of the two.