**Clostridium botulinum vaccine for veterinary use**

**Vaccinum clostridii botulini**
ad usum veterinarium

**DEFINITION**
Clostridium botulinum vaccine for veterinary use is prepared from a culture in liquid medium 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 in such a manner that toxicity is eliminated and immunogenic activity is retained.

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

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.

**IDENTIFICATION**
When injected into a healthy susceptible animal, the vaccine provokes the formation of specific antibodies against the type or types of *C. botulinum* from which the vaccine was prepared.

**TESTS**

**Safety.** Use 2 animals of one of the species for which the vaccine is intended and that have not been vaccinated against *C. botulinum*. Administer to each animal by a recommended route, twice the maximum dose stated on the label. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

**Residual toxicity.** Inject 0.5 ml of the vaccine subcutaneously into each of 5 mice, each weighing 17 g to 22 g. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

**Sterility.** It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

**POTENCY**
Use healthy white mice from a uniform stock, each weighing 18 g to 20 g. Use as challenge 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 of 50 per cent, a paralytic dose 50 per cent being the quantity of toxin which, when injected intraperitoneally 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 1 in 8 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 intraperitoneally 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 which show signs of botulism. All the control mice show signs of botulism during the observation period. The vaccine passes the test if not fewer than 80 per cent of the vaccinated mice are protected.

**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,

– that the preparation be shaken before use.

01/2005:0360

---

**Clostridium chauvoei vaccine for veterinary use**

**Vaccinum clostridii chauvoei**
ad usum veterinarium

**DEFINITION**
Clostridium chauvoei vaccine for veterinary use is prepared from a culture in liquid medium of one or more suitable strains of *Clostridium chauvoei*. The whole culture is inactivated in such a manner that toxicity is eliminated and immunogenic activity is retained. Inactivated cultures may be treated with a suitable adjuvant.

**IDENTIFICATION**
The vaccine protects susceptible animals against infection with *C. chauvoei*.

**TESTS**

**Safety.** Use 2 animals of one of the species for which the vaccine is intended and that have not been vaccinated against *C. chauvoei*. Administer to each animal at a single site, by a recommended route, twice the maximum dose stated on the label. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

**Sterility.** It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

**POTENCY**
Inject subcutaneously into not fewer than 10 healthy guinea-pigs, each weighing 350 g to 450 g, a quantity of the vaccine not exceeding the minimum dose stated on the label as the first dose. After 28 days, inject into the same animals a quantity of the vaccine not exceeding the minimum dose stated on the label as the second dose. 14 days after the second vaccination, inoculate intramuscularly into each

01/2005:0361
of the vaccinated guinea-pigs and into each of 5 control animals a suitable quantity of a virulent culture, or of a spore suspension, of C. chauvoei, activated if necessary with an activating agent such as calcium chloride. The vaccine complies with the test if not more than 10 per cent of the vaccinated guinea-pigs die from C. chauvoei infection within 5 days and all the control animals die from C. chauvoei infection within 48 h of challenge or within 72 h if a spore suspension was used for the challenge. If more than 10 per cent but not more than 20 per cent of the vaccinated animals die, repeat the test. The vaccine complies with the test if not more than 10 per cent of the second group of vaccinated animals die within 5 days and all of the second group of control animals die within 48 h of challenge or within 72 h if a spore suspension was used for the challenge. To avoid unnecessary suffering following virulent challenge, moribund animals are killed and are then considered to have died from C. chauvoei infection.

LABELLING

The label states that the preparation is to be shaken before use.

CLOSTRIDIUM NOVYI (TYPE B) VACCINE FOR VETERINARY USE

Vaccinum clostridii novyi B ad usum veterinarium

DEFINITION

Clostridium novyi (type B) vaccine for veterinary use is prepared from a liquid culture of a suitable strain of Clostridium novyi (type B).

PRODUCTION

The whole culture or its filtrate or a mixture of the two is inactivated in such a manner that toxicity is eliminated and immunogenic activity is retained. Toxoids and/or inactivated cultures may be treated with a suitable adjuvant, after immunogenic activity is retained. Toxoids and/or inactivated cultures may be treated with a suitable adjuvant, after concentration if necessary.

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 latter, it shall be demonstrated that for each target species the vaccine, when administered according to the recommended schedule, stimulates an immune response (for example, induction of antibodies) consistent with the claims made for the product.

BATCH TESTING

Residual toxicity. The test for residual toxicity may be omitted by the manufacturer, since a test for detoxification is carried out immediately after the detoxification process and, when there is risk of reversion, a second test is carried out at, as late a stage as possible during the production process.

Batch potency test. The test described under Potency is not necessarily carried out for routine testing of batches of vaccine. It is carried out for a given vaccine on one or more occasions as decided by or with the agreement of the competent authority. Where the test is not carried out, a suitable validated alternative test is carried out, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency and that has been shown to be satisfactory with respect to immunogenicity in the target species. The following test may be used after a satisfactory correlation with the test described under Potency has been established.

Vaccinate rabbits as described under Potency and prepare sera. Determine the level of antibodies against the alpha toxin of C. novyi in the individual sera by a suitable method such as an immunochemical method (2.7.1) or neutralisation in cell cultures. Use a homologous reference serum calibrated in International Units of C. novyi alpha antitoxin. Clostridia (multicomponent) rabbit antiserum BRP is suitable for use as a reference serum. The vaccine complies with the test if the level of antibodies is not less than that found for a batch of vaccine that has given satisfactory results in the test described under Potency and that has been shown to be satisfactory with respect to immunogenicity in the target species.

IDENTIFICATION

The vaccine stimulates the formation of novyi alpha antitoxin when injected into animals that do not have this antitoxin.

TESTS

Safety. Administer by a recommended route, to each of 2 sheep that have not been vaccinated against C. novyi (type B) twice the maximum dose of the vaccine stated on the label. Observe the animals for not less than 14 days. No abnormal local or systemic reaction occurs.

Residual toxicity. Inject 0.5 ml of the vaccine subcutaneously into each of 5 mice, each weighing 17 g to 22 g. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

Sterility. It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

POTENCY

Inject subcutaneously into each of not fewer than 10 healthy rabbits, 3 to 6 months old, a quantity of vaccine not exceeding the minimum dose stated on the label as the first dose. After 21 to 28 days, inject into the same animals a quantity of the vaccine not exceeding the minimum dose stated on the label as the second dose. 10 to 14 days after the second injection, bleed the rabbits and pool the sera. The potency of the pooled sera is not less than 3.5 IU/ml. The International Unit is the specific neutralising activity for C. novyi alpha toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The potency of the pooled sera obtained from the rabbits is determined by comparing the quantity necessary to protect mice or other suitable animals against the toxic effects of a fixed dose of C. novyi alpha toxin with the quantity of a reference preparation of Clostridium novyi alpha antitoxin, calibrated in International Units, necessary to give the same protection. For this comparison, a suitable preparation of C. novyi alpha toxin for use as a test toxin is required. The dose of the test toxin is determined in relation to the reference preparation; the potency of the serum to be examined is determined in relation to the reference preparation using the test toxin. Clostridium (multicomponent) rabbit antiserum BRP is suitable for use as a reference serum.