is not necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum virus titre stated on the label.

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**CLOSTRIDIUM BOTULINUM VACCINE FOR VETERINARY USE**

Vaccinum Clostridii botulini

ad usum veterinarium

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 liquid medium. Inactivated cultures may be treated with a suitable adjuvant.

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.

2.3. MANUFACTURER’S TESTS

2-3-1. Batch potency test. It is not necessary to carry out the Potency test (section 3-5) 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 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 and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

3-3. 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 recommended dose. 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. 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.

3-5. 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 which, 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 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 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 which show signs of botulism. The test is not valid unless all the control mice show no 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.

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.

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**CLOSTRIDIUM CHAUVOEII VACCINE FOR VETERINARY USE**

Vaccinum Clostridii chauvoei

ad usum veterinarium

1. DEFINITION

Clostridium chauvoeii vaccine for veterinary use is prepared from liquid cultures of one or more suitable strains of Clostridium chauvoei. The whole culture is inactivated to eliminate its toxicity while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for active immunisation of animals against disease caused by C. chauvoei.

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

C. chauvoei used for production is grown in an appropriate liquid medium. Inactivated cultures may be treated with a suitable adjuvant.

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.

3. BATCH TESTS

3-1. Identification. The vaccine protects susceptible animals against infection with C. chauvoei. The potency test may also serve for identification.
3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

3-3. **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 recommended dose. 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 not fewer than 10 healthy guinea-pigs, each weighing 350-450 g. Administer to each rabbit by the subcutaneous route a quantity of the vaccine not greater than the minimum dose stated on the label as the first dose. After 28 days, administer into the same animals a quantity of the vaccine not greater than the minimum dose stated on the label as the second dose. 14 days after the second vaccination, inoculate by the intramuscular route into each 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 euthanised and are then considered to have died from *C. chauvoei* infection.

2-2. **CHOICE OF VACCINE COMPOSITION.**

The vaccine is shown to be satisfactory with respect to safety (3.2.6) and efficacy (3.2.7) for the animals for which it is intended. 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.

2-3. **MANUFACTURER'S TESTS.**

2-3-1. **Residual toxicity.** 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 the stage as possible during the production process. The test for residual toxicity (section 3-4) may be omitted by the manufacturer.

2-3-2. **Batch potency test.** It is not necessary to carry out the Potency test (section 3-5) for each batch of 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 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 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 under Potency (section 3-5) 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 immunochromatographic 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 antisera 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.

3. **BATCH TESTS.**

3-1. **Identification.** The vaccine stimulates the formation of novyi alpha antitoxin when injected into animals that do not have this antitoxin.

3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

3-3. **Safety.** Administer by a recommended route, to each of 2 sheep that have not been vaccinated against *C. novyi* (type B) twice the maximum recommended dose of the vaccine. Observe the animals at least daily for not less than 14 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. **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.

3-5. **Potency.**

Use for the test not fewer than 10 healthy rabbits, 3-6 months old. Administer to each rabbit by the subcutaneous route.