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Clostridium Septicum Vaccine

General Notices

Braxy Vaccine

(Clostridium Septicum Vaccine for Veterinary Use, Ph. Eur. monograph 0364)

Ph Eur

1 DEFINITION

Clostridium septicum Vaccine for veterinary use is prepared from a liquid culture of a suitable strain of *Clostridium septicum*.

The whole culture or its filtrate (toxin) 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 and/or to protect passively their progeny against disease caused by *C. septicum*.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

C. septicum Used for production is grown in an appropriate liquid medium. Toxoid and/or 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. For the latter, it shall be demonstrated that for each target species the vaccine, when administered according to the schedule to be recommended, stimulates an immune response (for example, induction of antibodies) consistent with the claims made for the product.

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. septicum*. 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. If the test is carried out in pregnant animals, no adverse effects on gestation or the offspring are noted.

2-3 MANUFACTURER'S TESTS

2-3-1 Residual toxicity

Residual toxicity is assessed immediately after detoxification by a suitable *in vitro* method (e.g. in Vero cells). The result complies with the value specified for the product.

2-3-2 Antigen content

The antigen content is determined by a suitable *in vitro* method such as total combining power (TCP) using cells (e.g. Vero cells) as indicators of toxicity, an enzyme-linked immunosorbent assay (ELISA) or any other validated method.

2-3-3 Batch potency test

The potency test (section 3-3) is not carried out for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. 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 3-3) has been established.

Vaccinate rabbits as described under Potency and prepare sera. Determine the level of antibodies against the toxin of *C. septicum* 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 against *C. septicum* 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.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigenic component(s) of *C. septicum* stated under Definition.

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 Potency

Use for the test not fewer than 10 healthy rabbits, 3-6 months old. Administer to each rabbit by the subcutaneous route a quantity of vaccine not greater than the minimum dose stated on the label as the 1st dose. After 21-28 days, administer to the same animals a quantity of the vaccine not greater than the minimum dose stated on the label as the 2nd dose. 10-14 days after the 2nd injection, bleed the rabbits and pool the sera.

The vaccine complies with the test if the potency of the pooled sera is not less than 2.5 IU/mL.

The International Unit is the specific neutralising activity for *C. septicum* 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 Organization.

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 dose of *C. septicum* toxin with the quantity of a reference preparation of *Clostridium septicum* antitoxin, calibrated in International Units, necessary to give the same protection. For

this comparison, a suitable preparation of *C. septicum* 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.

Clostridia (multicomponent) rabbit antiserum BRP is suitable for use as a reference serum.

3-3-1 Preparation of test toxin. Prepare the test toxin from a sterile filtrate of a 1- to 3-day culture of *C. septicum* in liquid medium and dry by a suitable method. Select the test toxin by determining for mice the L+/5 dose and the LD_{50}, the observation period being 72 h.

A suitable toxin contains not less than 1 L+/5 dose in 1.0 mg and not less than 10 LD_{50} in each L+/5 dose.

3-3-2 Determination of test dose of toxin. Prepare a solution of the reference preparation in a suitable liquid so that it contains 1.0 IU/mL. Prepare a solution of the test toxin in a suitable liquid so that 1 mL contains a precisely known amount, such as 4 mg. Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each mixture contains 2.0 mL of the solution of the reference preparation (2 IU), one of a series of graded volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 5.0 mL. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 mice, each weighing 17-22 g, for each mixture, inject a dose of 0.5 mL by the intravenous or the intraperitoneal route into each mouse. Observe the mice for 72 h. If all the mice die, the amount of toxin present in 0.5 mL of the mixture is in excess of the test dose. If none of the mice die, the amount of toxin present in 0.5 mL of the mixture is less than the test dose. Prepare fresh mixtures such that 5.0 mL of each mixture contains 2.0 mL of the reference preparation (2 IU) and one of a series of graded volumes of the solution of the test toxin separated from each other by steps of not more than 20 per cent and covering the expected end-point. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 mice for each mixture, inject a dose of 0.5 mL by the intravenous or the intraperitoneal route into each mouse. Observe the mice for 72 h. Repeat the determination at least once and add together the results of the separate tests that have been made with mixtures of the same composition so that a series of totals is obtained, each total representing the mortality due to a mixture of a given composition.

The test dose of toxin is the amount present in 0.5 mL of that mixture which causes the death of one half of the total number of mice injected with it.

3-3-3 Determination of the potency of the serum obtained from rabbits

Preliminary test Dissolve a quantity of the test toxin in a suitable liquid so that 2.0 mL contains 10 times the test dose (solution of the test toxin). Prepare a series of mixtures of the solution of the test toxin and of the serum to be examined such that each contains 2.0 mL of the solution of the test toxin, one of a series of graded volumes of the serum to be examined and sufficient of a suitable liquid to bring the final volume to 5.0 mL. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 mice for each mixture, inject a dose of 0.5 mL by the intravenous or the intraperitoneal route into each mouse. Observe the mice for 72 h. If none of the mice die, 0.5 mL of the mixture contains more than 0.2 IU. If all the mice die, 0.5 mL of the mixture contains less than 0.2 IU.

Final test Prepare a series of mixtures of the solution of the test toxin and of the serum to be examined such that 5.0 mL of each mixture contains 2.0 mL of the solution of the test toxin and one of a series of graded volumes of the serum to be examined, separated from each other by steps of not more than 20 per cent and covering the expected end-point as determined by the preliminary test. Prepare further mixtures of the solution of the test toxin and of the solution of the reference preparation such that 5.0 mL of each mixture contains 2.0 mL of the solution of the test toxin and one of a series of graded volumes of the solution of the reference preparation to confirm the test dose of the toxin. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 mice for each mixture proceed as described in the preliminary test. The test mixture which contains 0.2 IU in 0.5 mL is that mixture which kills the same or almost the same number of mice as the reference mixture containing 0.2 IU in 0.5 mL. Repeat the determination at least once and calculate the average of all valid estimates. The test is valid only if the reference preparation gives a result within 20 per cent of the expected value.

The confidence limits (P = 0.95) have been estimated to be:

— 85 per cent and 114 per cent when 2 animals per dose are used;

— 91.5 per cent and 109 per cent when 4 animals per dose are used;

— 93 per cent and 108 per cent when 6 animals per dose are used.

4 LABELLING

The label states:

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

— for each target species, the immunising effect produced (for example, antibody production, protection against signs of disease or infection).
