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Clostridium Tetani Vaccines

General Notices

Tetanus Toxoids (Veterinary)

(Tetanus Vaccine for Veterinary Use, Ph. Eur. monograph 0697)

The name Clostridium Tetani Vaccine for Equidae (Tetanus Toxoid for Equidae) may be used for vaccines with an appropriate potency.

When Tetanus Toxoid is demanded for veterinary use, Clostridium Tetani Vaccine shall be supplied.

Ph Eur

1 DEFINITION

Tetanus vaccine for veterinary use is a preparation of the neurotoxin of *Clostridium tetani* inactivated to eliminate its toxicity while maintaining adequate immunogenic properties. The vaccine may be used to induce active and/or passive immunity.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

C. tetani Used for production is grown in an appropriate liquid medium. The toxin is purified and then detoxified or it may be detoxified before purification. The detoxification process is validated to demonstrate its ability to consistently produce a toxoid that is immunogenic and stably detoxified, including at the concentration used in the final lot.

The antigenic purity is determined in Lf units of tetanus toxoid per milligram of protein and shown to be not less than the value approved for the particular product.

The toxin content (Lf per millilitre) is checked (2.7.27) to monitor consistency of production.

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 tests for production of antigens (section 2-2-1), safety (section 2-2-2) and immunogenicity (section 2-2-3) may be used during demonstration of safety and efficacy.

The *C. tetani* strain used in the preparation of the vaccine is shown to be satisfactory with respect to the production of the neurotoxin.

2-2-1 Production of antigens

The production of the neurotoxin of *C. tetani* is verified by a suitable immunochemical method (2.7.1) carried out on the neurotoxin obtained from the vaccine strain under the conditions used for the production of the vaccine.

2-2-2 Safety

Carry out the test 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 and of the most sensitive category for the species. 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, free from antitoxic antibodies. Administer to each animal 1 dose of 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 at least 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.

2-2-3 Immunogenicity

2-2-3-1 Immunogenicity test in the target species

It shall be demonstrated for each target species that the vaccine, when administered according to the schedule to be recommended and by the route to be recommended, stimulates an immune response (for example, induction of antitoxic antibodies or induction of protective levels of antitoxic antibodies) consistent with the claims made for the product.

2-2-3-2 Immunogenicity test in guinea-pigs

Administer 1 dose of vaccine by the subcutaneous route to each of at least 5 guinea-pigs that do not have antibodies against the neurotoxin of *C. tetani*. After 28 days, administer again to each guinea-pig 1 dose by the subcutaneous route. 14 days after the 2nd dose, collect blood from each guinea-pig and prepare serum samples. Determine for each serum the titre of antibodies against the neurotoxin of *C. tetani* using a suitable immunochemical method (2.7.1) such as a toxin-binding-inhibition test (ToBI test) and a homologous reference serum. Determine the average antibody titre of the serum samples.

Clostridium tetani guinea-pig antiserum for vaccines for veterinary use BRP is suitable for use as a reference serum.

Tetanus vaccine intended for use in animals other than horses complies with the test if the average antibody titre is not less than 7.5 IU/mL.

Tetanus vaccine intended for use in horses complies with the test if the average antibody titre is not less than 30 IU/mL.

For tetanus vaccine presented as a combined vaccine for use in animals other than horses, the above test may be carried out in susceptible rabbits instead of guinea-pigs. The vaccine complies with the test if the average antibody titre of the sera of the vaccinated rabbits is not less than 2.5 IU/mL.

Clostridia (multicomponent) rabbit antiserum BRP and *Clostridium tetani rabbit antiserum BRP* are suitable for use as reference sera.

2-3 MANUFACTURER'S TESTS

2-3-1 Absence of tetanus toxin

Inject subcutaneously 1 mL containing at least 500 Lf of bulk purified toxoid into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the toxoid does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the toxoid does not comply with the test.

2-3-2 Batch potency test

It is not necessary to carry out the potency test (section 3-3) 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.

Where the test described under Potency is used as the batch potency test, the vaccine complies with the test if the antibody titre in International Units is not less than that found for a batch of vaccine shown to be satisfactory with respect to immunogenicity in the target species.

3 BATCH TESTS

3-1 Identification

If the nature of the adjuvant allows it, carry out test A. Otherwise carry out test B.

A. Dissolve in the vaccine sufficient *sodium citrate R* to give a 100 g/L solution. Maintain the solution at 37 °C for about 16 h and centrifuge until a clear supernatant is obtained. The supernatant reacts with a suitable tetanus antitoxin, giving a precipitate.
B. When injected into animals that do not have antibodies against the neurotoxin of *C. tetani*, 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)*.

3-43 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-3-2).
