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

Clostridium Tetani Antitoxin

General Notices

Tetanus Antitoxin (Veterinary)

(Tetanus Antitoxin for Veterinary Use, Ph. Eur. monograph 0343)

Ph Eur

DEFINITION

Tetanus antitoxin for veterinary use is a preparation containing principally the globulins that have the power of specifically neutralising the neurotoxin formed by *Clostridium tetani*. It consists of the serum or a preparation obtained from the serum of animals immunised against tetanus toxin.

PRODUCTION

CHOICE OF COMPOSITION

The antitoxin is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*). For the latter, it shall be demonstrated, for each target species, that the product, when administered at the minimum recommended dose and according to the recommended schedule(s), provides a response or responses consistent with the claims made for the product. The ability of the product to neutralise the neurotoxin formed by *C. tetani* must also be demonstrated, e.g. by conducting the test in mice as described below.

Demonstration of neurotoxin neutralisation

The ability of tetanus antitoxin to neutralise the neurotoxin of *C. tetani* is determined by establishing the dose necessary to protect mice (or guinea-pigs) against the toxic effects of a fixed dose of tetanus toxin. The test must be conducted in parallel with a test of a reference preparation of tetanus antitoxin, calibrated in International Units, using a quantity expected to give the same protection. The ability of the test antitoxin to neutralise the neurotoxin (potency) can then be expressed in International Units. For this study, a suitable preparation of tetanus 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 antitoxin to be examined is determined in relation to the reference preparation using the test toxin.

Preparation of test toxin

Prepare the test toxin from a sterile filtrate of an 8-10 day culture in liquid medium of *C. tetani*. A test toxin may be prepared by adding this filtrate to *glycerol R* in the proportion of 1 volume of filtrate to 1 to 2 volumes of *glycerol R*. The solution of test toxin may be stored at or slightly below 0 °C. The toxin may also be dried by a suitable method. Select the test toxin by determining for mice the Lp/10 dose and the paralytic dose 50 per cent. A suitable toxin contains not less than 1000 times the paralytic dose 50 per cent in 1 Lp/10 dose.

Lp/10 dose (Limes paralyticum)

This is the smallest quantity of toxin which when mixed with 0.1 IU of antitoxin and injected subcutaneously into mice (or guinea-pigs) causes tetanic paralysis in the animals on or before the 4th day after injection.

Paralytic dose 50 per cent

This is the quantity of toxin which when injected subcutaneously into mice (or guinea-pigs) causes tetanic paralysis in one half of the animals on or before the 4th day after injection.

Determination of test dose of toxin

Reconstitute or dilute the reference preparation with a suitable liquid so that it contains 0.5 IU/mL. Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid. Prepare mixtures of the solution of the reference preparation and the solution of the test toxin so that each mixture will contain 0.1 IU of antitoxin in the volume chosen for injection 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. Adjust each mixture with a suitable liquid to the same final volume (0.4 mL to 0.6 mL if mice are used for the test or 4.0 mL if guinea-pigs are used). Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 animals for each mixture, inject the chosen volume subcutaneously into each animal. Observe the animals for 96 h and make daily records of the degree of tetanus developing in each group of animals. Repeat the test at least once and calculate the test dose as the mean of the different tests. The test dose of the toxin is the amount present in that mixture which causes tetanic paralysis in one half of the total number of animals injected with it.

Determination of the neutralising ability of the antitoxin to be examined

Preliminary test Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid so that the solution contains 5 test doses per millilitre (solution of the test toxin). Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined so that for each mixture the volume chosen for injection contains the test dose of toxin and one of a series of graded volumes of the antitoxin to be examined. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 animals for each mixture, inject the chosen volume subcutaneously into each animal. Observe the animals for 96 h and make daily records of the degree of tetanus developing in each group of animals. Using the results, select suitable mixtures for the final test.

Final test Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined so that for each mixture the volume chosen for the injection contains the test dose of toxin and one of a series of graded volumes of the antitoxin to be examined, separated from each other by steps of not more than 20 per cent and covering the expected end-point as determined in the preliminary test. Prepare further mixtures with the same amount of test toxin and graded volumes of the reference preparation, centred on 0.1 IU in the volume chosen for injection, to confirm the test dose of the toxin. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than 2 animals for each mixture, inject the chosen volume subcutaneously into each animal. Observe the animals for 96 h and make daily records of the degree of tetanus developing in each group of animals. The test mixture which contains 0.1 IU in the volume injected is that mixture which causes tetanic paralysis in the same, or almost the same, number of animals as the reference mixture containing 0.1 IU in the volume injected. Repeat the determination at least once and calculate the mean of all valid estimates. Estimates are 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 3 animals per dose are used;

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

IDENTIFICATION

The antitoxin is shown, by a suitable immunochemical method (2.7.1), to react specifically with the neurotoxin formed by *C. tetani*. The potency test may also serve for identification.

POTENCY

Determine the titre of antibodies against the neurotoxin formed by *C. tetani* using a suitable immunochemical method (2.7.1) such as a toxin-binding-inhibition test (ToBI test) and a homologous reference serum, calibrated in International Units per millilitre.

The International Unit is the specific neutralising activity for tetanus 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 finished product is expressed in International Units per millilitre and is shown to be not less than the minimum number stated on the label.

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