DRAFT REVISED MONOGRAPH FOR COMMENTS

This draft revised monograph contain text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

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Tetanus Veterinary Vaccine

Tetanus Veterinary Vaccine is a preparation of the neurotoxin of *Clostridium tetani* treated in a manner that eliminates toxicity while maintaining adequate immunogenic properties. This monograph is intended for the vaccination of cattle, horses, sheep, goats and swine against tetanus infection.

Production

The *C. tetani* strain used for production is cultured in a suitable medium and a facility having tetanus vaccine for human use can be sourced for further production processes. 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.

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

Choice of vaccine composition

The *C. tetani* strain used in the preparation of the vaccine is shown to be satisfactory with respect to the production of the neurotoxin. The vaccine is shown to be satisfactory with respect to safety and immunogenicity for each species of animal for which it is intended. As part of the studies to demonstrate these characteristics, the tests described below may be used.

Bulk Purified Toxoid

Absence of tetanus toxin

Inject subcutaneously at least 500 Lf of purified toxoid in a volume of 1 ml into each of five healthy guinea pigs, each weighing 250 to 350 g, that have not previously been treated with any material that will interfere with the test. The bulk purified toxoid passes the test if none of the animals shows any symptoms of tetanus toxaemia or dies from tetanus within 21 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight repeat the test. If an animal dies or loses weight in the second test the toxoid fails the test.

Irreversibility of toxoid

Carry out a test for reversion to toxicity on the detoxified harvest using 2 groups of 5 guinea-pigs, each weighing between 350 to 450 g; if the vaccine is adsorbed, carry out the test with the shortest practical time interval before adsorption. Prepare a dilution of the detoxified harvest so that the guinea-pigs each receive 10 times the amount of toxoid (measured in Lf units) that will be present in a dose of vaccine. Divide the dilution into 2 equal parts. Keep one part at 5 ± 3° and the other at 37° for 6 weeks. Attribute each dilution to a separate group of guinea-pigs and inject into each guinea-pig the dilution attributed to its group. Observe the animals for 21 days. The toxoid complies with the test if no guinea-pig shows clinical signs of disease or dies from causes attributable to the neurotoxin of *C. tetani*.

Master seed lot

The Master seed lot of *C. tetani* is maintained at recommended storage-temperatures. Production of the neurotoxin of *C. tetani* on the neurotoxin obtained from the vaccine strain under the condition used for the production of the vaccine. The master seed lot complies

with the tests of purity and identity for the organism and a batch of vaccine prepared from the master seed lot should comply with full range of control tests, i.e. identification, safety and potency. For identification, molecular approaches are acceptable.

Identification.

Carry out test A if permitted by the nature of the adjuvant. Otherwise carry out test B.

A. Separate the toxoid from the adjuvant. For vaccines adsorbed on aluminium hydroxide, the following treatment is suitable. Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. The clear supernatant liquid reacts with a suitable tetanus antitoxin and yields a precipitate.

B. When inoculated into healthy susceptible animals, the vaccine stimulates the formation of antitoxin to the neurotoxin of *C. tetani* or protects the animals against the paralytic effects of the toxin. When identification test is carried out on master seed or working seed, it can be omitted as a routine test on in process or finished product provided traceability is established.

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.

Immunogenecity.

Determine by either method A or method B

A. Inject subcutaneously to each of at least five guinea pigs, each weighing between 350 and 450 g, with a quantity of the vaccine not more than the minimum dose stated on the label as the primary dose, and 28 days later with a quantity of the vaccine not more than the minimum dose stated on the label as the secondary dose. Fourteen days after the second dose, collect the blood from each guinea pig, pool the sera and determine the antitoxin titre by the biological assay of *C. tetani* antitoxin described below. 1 ml of serum contains not less than 7.5 IU per ml or, for vaccine intended for use in equine, not less than 30 IU per ml. When *C. tetani* vaccine is presented as a component-of a mixed vaccine intended for use in animals other than equine and the potency test of the other component or components normally carried out using rabbits, the potency test described above may be carried out using ten healthy rabbits, between 3 and 6 months old. 1 ml of serum contains not less than 2.5 Units.

Biological assay of *C. tetani* antitoxin

The potency of *C. tetani* antitoxins is determined by comparing the dose necessary to protect mice or other suitable animals against the toxic effects of a fixed dose of *C. tetani* toxin with the quantity of a standard preparation of *C. tetani* antitoxin necessary to give the same protection. For this purpose, the standard preparation of *C. tetani* antitoxin and a suitable preparation of *C. tetani* toxin are required. The test dose of the toxin is determined in relation to the standard preparation of antitoxin and the potency of the preparation under examination is then determined in relation to the standard preparation using the test toxin.

***C. tetani* antitoxin standard preparation**

It is recommended to obtain the international standards or reference standard from any recognized international laboratories or any other suitable preparation, the potency of which has been determined in relation to the international standard method.

Suggested method

NOTE— The severity of tetanic paralysis to be regarded as the end-point is such that the paralysis is readily recognized but not sufficiently extensive to cause significant suffering.

In practice, when using high levels of toxin to determine the test dose, or when using low levels of antitoxin in the preliminary and final tests, the development of paralysis is so rapid that the defined end-point is usually synchronous with death. Where death occurs, the combined totals of animals dying or reaching the paralytic end-point are used in the calculations.

Preparation of test toxin.

Prepare *C. tetani* toxin by growing *C. tetani* in liquid culture for 8 to 10 days and then adding 1 volume of a sterile filtrate of the culture to 1 or 2 volumes of *glycerine*. Store at 0° or at temperatures slightly below it. The toxin may be dried by a suitable method.

Selection of test toxin.

Select toxin for use as the test toxin by determining the following quantities:

LP/10 dose (Limes paralyticum).

This is the smallest quantity of toxin that when mixed with 0.1 Unit of antitoxin and injected subcutaneously into mice (or guinea pigs) causes titanic paralysis in the animals on or by the fourth day after injection.

Paralytic dose 50.

This is the quantity of toxin that when injected subcutaneously into mice (or guinea pigs) cause tetanic paralysis in one-half of the animals injected on or by the fourth day after injection. A suitable toxin is one that contains not less than 1000 paralytic dose 50 in an LP/10 dose.

Determination of test dose of toxin.

Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid. Reconstitute or dilute the Standard preparation with a suitable liquid to give a solution containing 0.5 Unit in 1 ml. Prepare mixtures of the solution of the Standard preparation and the solution of the test toxin such that each mixture contains 0.1 Unit of antitoxin in the volume selected for injection and one of a series of graded volumes of the solution of the toxin, separated from each other by steps of not more than 20 per cent and covering the expected end-point. Adjust each mixture to the same final volume (0.4 to 0.6 ml if mice are used or 4.0 ml if guinea-pigs are used) with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject a dose if-the selected volume of each mixture subcutaneously into each of not less than 2 animals of the group to which each mixture has been allocated. Observe the animals for 4 days and record daily the degree of tetanus developing in each group of animals. Repeat the determination at least once, add together the results of the separate tests that have been made with mixtures of the same composition such that a series of totals is obtained and determine the mean values. The test dose of the toxin is the amount present in that mixture that causes tetanic paralysis in one-half of the total number of animals injected with it. When the test dose of the test toxin has been determined, a concentrated solution of the test toxin may be prepared in a mixture consisting of 1 volume of *saline solution* and 1 or 2 volumes *of glycerine.* This concentrated solution may be stored frozen and diluted as required. The specific activity of such a solution must be determined at frequent intervals.

Determination of potency of the antitoxin

Preliminary test. Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid such that the solution contains 5 test doses per ml. Prepare mixtures of the solution of the test toxin and the preparation under examination such that for each mixture the volume selected for injection contains the test dose of toxin and one of a series of graded volumes of the preparation under

examination. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes. Inject a dose of the selected volumes of each mixture subcutaneously into each of not less than

two animals of the group to which each mixture has been allocated. Observe the animals for 4 days and record daily the degree of tetanus developing in each group of animals. From the results select suitable mixtures for the final test.

Final test. Prepare similar fresh mixtures of the test toxin and the preparation under examination such that for each mixture the volume selected for injection contains the test dose of toxin and one of a series of graded volumes of the preparation under examination, 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 Standard preparation, centered on 0.1 Unit in the volume selected for injection to confirm the test dose of the toxin. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixture to stand at room temperature, protected from light, for 60 minutes. Inject a dose of the selected volume of each mixture subcutaneously into each of not less than two animals of the group to which each mixture has been allocated.
Observe the animals for 4 days and record daily the degree of tetanus developing in each group of animals. The mixture of antitoxin under examination that contains 0.1 Units in the volume injected is that mixture which causes tetanic paralysis in the same, or almost the same number of animals as the mixture containing 0.1 Unit of the Standard preparation in the volume injected. Repeat the determination at least once and calculate the average of all valid estimates. Estimates are not valid unless the Standard preparation gives a result within 20 per cent of the expected value.

Limits of error. For the suggested method, the limits of error (P = 0.95) have been estimated to be 85 to 114 per cent when two animals are used for the test, 91.5 to 109 percent when three animals are used and 93 to 108 percent when six animals are used per the dose.

B. Carry out the biological assay of adsorbed tetanus vaccine as stated under Tetanus Vaccine (Adsorbed).
This method may only he used for those preparations for which it has been shown to be suitable and in particular may not be suitable for vaccine with an oil adjuvant. Where this alternative method is used the estimated potency is not less than 150 IU in the smallest dose stated on the label.

Manufacturer's Tests

Absence of tetanus toxin
The vaccine complies with the test for Absence of tetanus toxin mentioned under section of production.

Irreversibility of toxoid
The vaccine complies with the test for Absence of tetanus toxin mentioned under section of production.

Potency test
The vaccine complies with the requirements of the test or test(s) mentioned under immunogenicity when administered by a recommended route and method.

Batch Tests

Identification. Vaccine complies with the requirements of the tests mentioned under production. Alternatively suitable validated immunochemical/ molecular biology methods can be used in the routine batch release tests after proper validation (2.8.1).

Bacterial and fungal Contamination. The vaccine, including where applicable the diluent supplied for reconstitution complies with the test for sterility (2.2.11).

Residual toxicity. Administer 5 mL of the vaccine by the subcutaneous route as 2 equal divided doses at separate sites, into each of 5 healthy guinea-pigs, each weighing 350-450 g, that have not previously been treated with any material that will interfere with the test. The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test. If any animal dies in the 2nd test, the vaccine does not comply with the test.

Potency

The vaccine complies with the requirements of the test or test(s) mentioned under immunogenicity when administered by a recommended route and method.

Labelling

The label must state that (1) the vaccine is for veterinary use only; (2) the recommended routes of administration; (3) the instructions for use, such as – "the preparation should be shaken well before use or reconstituted with the diluent supplied for reconstitution where applicable"; (4) the animal species for which the vaccine is intended; (5) storage temperatures; (6) Batch Number, Manufacturing date and expiry date; (7) Precautions in pregnant [animals] (If applicable) (8) Total volume and number of doses; (9 the name of the adjuvant used; 10) Strain of bacterium used for vaccine production; 11) the minimum units per single dose