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.	

Enterotoxaemia Vaccine, Inactivated

Clostridium welchii Type D Vaccine, *Clostridium perfringens* Type D Vaccine, Pulpy Kidney Vaccine

Enterotoxaemia Vaccine, Inactivated is a culture of highly toxigenic strain of *Clostridium perfringens* Type D grown in an anaerobic medium and rendered sterile and non-toxic by the addition of a suitable quantity *of formaldehyde* in such a manner that it retains its immunizing properties. The toxoid and/or inactivated culture may contain a suitable adjuvant. This monograph applies to the vaccines intended for active immunization of animals against enterotoxaemia caused by *C. perfringens* Type D.

Production

Preparation of vaccine.

Selected toxigenic *C. perfringens* Type D strain used for production is grown in a suitable anaerobic fluid medium under conditions which ensure maximum epsilon(ε) toxin production. The culture is tested for purity and trypsinized to activate the ε prototoxin. The epsilon (ε) toxin titer is determined by mice inoculation. Solution of *formaldehyde* is added in a suitable concentration and the formalized culture is kept at 37° till the product is sterile and non-toxic. A suitable adjuvant may be added.

Choice of vaccine strain and composition

A reference, highly toxigenic strain of *C. perfringens* Type D, obtained from an authentic source should be used. The vaccine contains a highly toxigenic, inactivated strain of *C. perfringens* Type D with or without a suitable adjuvant. The vaccine is shown to be satisfactory with respect to safety (*2.7.17*) and efficacy (*2.7.12*) 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.

Safety and Immunogenicity

At least 8 sheep each weighing not less than 18 kg are used for testing safety and potency of master seed lot. Each of two sheep receives subcutaneously 10 ml of the test product. Each of the remaining six sheep receives 2.5 ml of the test product through subcutaneous route. The animals are observed for 5 days.

The product passes the safety test if only a minimum of local reaction and no systemic reaction is observed in the animals. Sheep receiving 10 ml of the product are withdrawn from the experiment after 5 days.

Inoculate each of the remaining 6 sheep with a second dose of 2.5 ml after an interval of 14 to 21 days of first inoculation. Bleed the animals 10 to 14 days after the second dose and determine the ε antitoxin titre in the pooled serum sample by testing on mice as follows.

1 ml of the pooled serum is mixed with 1.0 ml of epsilon (ε)toxin of *C. perfringens* Type D, containing 300 mouse minimum-lethal doses (mouse m.l.d.) and kept at room temperature for 30 minutes. At least 2 mice each weighing not less than 18 g are each injected intravenously 0.2 ml of the mixture. Each of two control mice, each weighing not less than 18 g receive 0.2 ml of toxin containing 300 mousem.l.d. per ml diluted with equal volume of *normal saline*. The control mice should die within 1 to 2 hours while the mice receiving the mixture of serum and toxin should survive for at least 2 days. Serum containing one International Unit (IU) of ε antitoxin per ml will be able to neutralize 150 mouse m.l.d. of ε toxin of *C. perfringens* Type D.

The product passes the test if the post inoculation pooled sheep serum contains not less than 2 IU of ε antitoxin per ml.

Manufacturer's tests

Safety and potency. The tests may be carried out on rabbits. Use at least 12 rabbits each weighing not less than 1 kg. Each of the rabbit is immunized with 5 ml of the preparation through subcutaneous route. The animals are observed for 5 days during which they should not show any systemic reaction. Only a minimum local reaction may be observed. After one month, each of the animals is inoculated with second dose of 5 ml of the product through the same route. Bleed the animals 10 to 14 days after the second dose and determine the Ɛ antitoxin titre in the pooled serum sample by testing on mice as described for sheep. The product passes the test if the post-inoculation pooled sheep serum contains not less than 2 IU of Ɛ antitoxin per ml.

Residual live bacteria/ toxins. A suitable validated method for complete inactivation of residual live bacteria/ toxins shall be used with the approval of competent authority.

Batch tests

Description. Off white to Yellowish-brown liquid containing dead bacteria in suspension.

Identification. When injected into susceptible animals, the vaccine stimulates production of Ɛ antitoxin of *C. perfringens* Type D. The potency test may also serve for identification.

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

Safety and Potency. The vaccine complies with the test for safety and potency mentioned under section of Manufacturer's tests/Production.

Note: General Requirements shall be referred regarding omission of the batch safety test.

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) strain of bacterium used for the preparation of vaccine