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.	

Blackquarter Vaccine

Blackleg Vaccine; Clostridium chauvoei Vaccine Inactivated

Blackquarter Vaccine is a liquid culture of a suitable strain or strains of *Clostridium chauvoei* grown in a suitable anaerobic fluid medium and rendered sterile and non-toxic by addition of *formaldehyde* in such a manner that it retains its immunizing properties. The vaccine may contain a suitable adjuvant. This monograph applies to the vaccines intended for active immunization of animals against disease caused by *C. chauvoei.*

Production

Preparation of vaccine. *C. chauvoei* strain used for production is grown in a suitable anaerobic fluid medium and the whole culture is inactivated by addition of suitable quantity of *formaldehyde.* The inactivated bacterial cultures may be partially purified and concentrated to reduce the components of spent media during manufacturing of bulk antigen (drug substance). A suitable adjuvant may be added to the inactivated cultures.

Choice of vaccine strain and vaccine composition. A reference strain of *C. chauvoei* obtained from an authentic source should be used. However, a local isolate from a particular area may also be used if the strain is shown to be satisfactory with respect to safety and immunogenicity for the animals for which the vaccine is intended. The vaccine contains inactivated strain or strains of immunogenic *C. chauvoei* with or without a suitable adjuvant. The vaccine is shown to be satisfactory with respect to identification, safety and immunogenicity for the animal species for which it is intended.

Master Seed Lot.

The seed lot should comply with the tests for purity and identity and, a batch of vaccine prepared from the seed lot should comply with entire range of control tests as mentioned below.

Identification. Identification of the bacteria is demonstrated by means of morphological, immunological or molecular methods. A suitable molecular method such as polymerase chain reaction can be used to establish the identity of bacterial strain. The vaccine protects susceptible animals against infection with *C. chauvoei.* The potency test may also serve for identification.

Safety. Carry out safety test either on sheep or on cattle as per following procedure.

Sheep. Inoculate three one-year-old sheep not vaccinated against Blackquarter vaccine with double the dose of the vaccine by recommended route of administration at the inner face of the thigh. Observe for 10 days and record their temperature in the morning and evening.

Cattle. Inoculate three one year old cattle not vaccinated against black quarter vaccine with double the recommended dose of the vaccine by recommended route at the neck region. Observe the inoculated animals for 10 days and record their temperature twice a day in the morning and evening.

The seed lot passes the test if there is no untoward reaction except slight swelling at the site of inoculation which subsides in four to five days.

Immunogenicity. Inoculate each of ten healthy guinea-pigs weighing between 350 grams and 450 grams subcutaneously with 2 ml of the vaccine or a quantity of the vaccine not greater than the minimum dose stated on the label as a primary dose. After 7 days, re-inoculate the same animals with 2 ml of the vaccine or a quantity of the vaccine not greater than the minimum dose stated on the label as a secondary dose. None of the vaccinated guinea-pigs shows any systemic reaction**.** A minimal local reaction may be observed in the animals. Fourteen days after the second vaccination, challenge all vaccinated guinea-pigs along with five controls by intramuscular route with a suitable quantity of virulent culture or spore suspension *in* saline suspension containing 2.0 per cent *calcium chloride.*

The vaccine complies with the test if not more than 10 per cent of the vaccinated guinea-pigs die from *C. chauvoei* infection within 7 days, and all control guinea-pigs die from the C. *chauvoei* infection within 72 hours of challenge. If more than 10 per cent but less than 20 per cent of the vaccinated animals die, repeat the test. The vaccine complies with the test if not more than 10 per cent of the second group of vaccinated guinea-pigs die from the *C. chauvoei* infection within 7 days, and all of the animals of the control group die from the C. *chauvoei* infection within 72 hours of challenge. To avoid unnecessary sufferings following virulent challenge, moribund animals are euthanized and are considered to have died from *C. chauvoei* infection.

Manufacturer's tests

Identification. The vaccine complies with the requirements of the test mentioned under section of master seed lot.

Bacterial and Fungal Contamination (2.2.11)**.** The bulk shall not contain contaminating bacteria and fungi and shall comply with the requirements of the test for sterility.

Safety and potency. Each lot of at least 6 healthy adult guinea-pigs weighing between 350 grams and 450 grams is injected subcutaneously with recommended dose of the vaccine followed by a second injection 2 weeks later with the same dose of vaccine. Observe the vaccinated guinea pigs for any systemic or a minimal local reaction**.** Minimal local reaction may be observed. Fourteen days after the second vaccination, challenge all vaccinated guinea-pigs along with 2 controls by intramuscular route with 20 viable spores or with 1 MLD (Minimal Lethal Dose) of virulent C. *chauvoei in* saline suspension containing 2 per cent *calcium chloride.*

The vaccine complies with the test if at least 4 of the 6 vaccinated guinea-pigs survive from the *C. chauvoei* infection for 7 days, and the two control guinea-pigs die from the infection within 48 hours of challenge.

Test for residual live bacteria. Carry out either the test A or B

A. Inject each of not less than 5 healthy mice weighing 18 to 20 g by intramuscular route with 0.1 ml of the inactivated antigen. Observe the animal for 7 days. None of the mice dies or shows any abnormalities attributable to the vaccine.

B. Inject each of not less than 2 healthy guinea pigs weighing 350 to 450 g by intramuscular route with 0.2 ml of the inactivated antigen. Observe the animal for 7 days. None of the guinea pigs dies or shows any abnormalities attributable to the vaccine.

Batch tests

Description. An off-white to yellowish-brown liquid containing dead bacteria in suspension.

Identification. The vaccine complies with the requirements of the test mentioned under section of master seed lot.

Bacterial and Fungal contamination (2.2.11)**.** Complies with the test for sterility.

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

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 dose and routes of administration; (3) the instructions for use, such as – "the preparation should be shaken well before use; (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 strain or strains used in vaccine preparation.