# 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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# Haemorrhagic Septicaemia Vaccine, Inactivated

Haemorrhagic Septicaemia Vaccine, Inactivated, is a preparation of *Pasteurella multocida.* The whole culture is inactivated by *formaldehyde* and a suitable adjuvant is added. This monograph applies to the vaccines intended for active immunization of animals against disease caused by *Pasteurella multocida.*

## Production

**Preparation of Vaccine.** Pure suspension of a highly immunogenic strain of *Pasteurella multocida* grown in phase I on a suitable medium by a suitable method (Agar wash or Fermenter) is inactivated by a suitable quantity of *formaldehyde.* The inactivated bacterial cultures may be purified and concentrated to reduce the components of spent media during manufacturing of bulk antigen (drug substance). The suspension is adjusted to a desired Brown's opacity scale or any other suitable method before addition of adjuvant (Alum or Aluminium hydroxide gel or oil adjuvant) so that the finished product complies with the tests for identification, safety and immunogenicity for the animal species for which it is intended.

**Choice of vaccine strain and composition.** A reference strain of *Pasteurella multocida,* obtained from an authentic source is used**.** However, a suitable 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.

### Master seed lot

The master seed lot of the vaccine strain of *P. multocida* is not more than 1 passage on an artificial medium from the culture obtained after target animal passage. The master seed lot complies with the tests *of purity* and *identity* for the organism and a representative batch of vaccine prepared from the master seed lot complies with full range of control tests, i.e. identification, safety and immunogenicity or potency.

**Antigenic mass.** The following method is suggested for adjusting the antigenic mass. Centrifuging at least 100 ml of the final inactivated bulk suspension in each of 4 pre-weighed (up to milligram level) centrifuge tubes at 5000 rpm for 30 minutes. Discard supernatant and, dry the pellet by an appropriate method. Determine the dry weight of the pellets in the tubes. Calculate volume *of phenol saline* to be added to the bulk so that dry weight of the cell mass is between 140 to 150 mg per 100 m1.

**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 *P. multocida*. The potency test may also serve for identification.

**Safety.**

*A. Safety in bovines.* Inject at least 2 apparently healthy buffalo or male cow claves with twice the dose of the product through appropriate route (subcutaneous for Alum and gel or intramuscular for oil adjuvant) and observe for 10 days. The master seed lot passes the safety test if none of the animal shows any obvious adverse reaction and die of *P. multocida* infection.

*B. Safety in mice.* Inject intraperitoneally into each of six healthy mice weighing not less than 18 g with 0.5ml of the preparation under test and observe for 5 days. No abnormal reaction occurs and none of the mice dies of *P. multocida* infection.

**Immunogenicity.**

*A. Immunogenicity in bovines.* Use 3 apparently healthy buffalo or male cow calves which have been tested free from anti-*P. multocida* antibodies or have not been vaccinated earlier with the said vaccine and age between 6 months - 2 years. Keep two more such animals as controls. Inoculate 2ml (animals having body weight less than 140 kg) or 3ml (animals having body weight more than 140 kg) of the test product from 5 pooled samples through route recommended for the vaccine. (Inject alum or gel vaccine through subcutaneous and oil adjuvant vaccine through intramuscular route). Challenge the vaccinated animals along with two healthy controls with at least 50 million mouse minimum lethal dose of a virulent *P. multocida* culture after 21 days. Observe the animals for 7 days. The master seed lot passes the immunogenicity test if both the controls die of Haemorrhagic septicaemia and at least 2 out of the 3 vaccinated, survive the challenge.

*B. Immunogenicity in mice.* Inject fifty mice of either sex weighing not less than 18 g, subcutaneously with 0.2 ml of alum or gel vaccine or intramuscularly with 0.2 ml of the oil adjuvant vaccine from 5 pooled samples. Repeat the dose similarly after 14 days. After 7 day of the second vaccination divide the vaccinated mice into 10 groups of 5 each. Use fifty mice of the same from the same stock as controls divided similarly into 10 groups of 5 each: Challenge each of the vaccinated and the control mice with 0.2 ml of a dilution of 12 to 18 hours old broth culture of a virulent strain of *P. multocida* ranging from $10^{-1}$ to $10^{-10}$ through subcutaneous route. Observe the mice for 5 days and record the mortalities in vaccinated and control groups. Calculate the 50 per cent lethal dose of the challenge organism for vaccinated and control mice by Spearman and Karber method.

The protection provided by the vaccine is determined as Protective Index (PI), by using following formula:
Protective Index (PI) = $LD_{50}$ in control mice / $LD_{50}$ in vaccinated mice
The vaccine passes the test if it provides a minimum PI of 4 $\log_{10}$.

**Manufacturer's Tests**

**Identification**. Complies with the requirements of the test mentioned under section of master seed lot.

**Sterility** (2.2.11).The bulk shall not contain contaminating bacteria and fungi and, shall comply with the requirements of the sterility test mentioned under general requirements.

**Potency.** The vaccine complies with requirements of either of the immunogenicity test mentioned under section of master seed lot.

## Batch tests

**Description.** An off-white to yellowish liquid containing dead bacteria in suspension or it appears as off-white emulsion, if mineral oil adjuvant is used in the vaccine preparation.

**Identification.** 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.** The vaccine complies with the requirements of the test mentioned under section of Master seed lot.
*Note: General Requirements shall be referred regarding omission of the batch safety test.*

**Potency.** The vaccine complies with the requirement of either of the immunogenicity tests mentioned under section of master seed lot.

**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; (4) the animal species for which the vaccine is intended; (5) Batch Number, Manufacturing date and expiry date; (6) Total volume and number of doses; (7) adjuvant used; (8) the strain used in vaccine preparation.