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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Brucella abortus (Strain 19) Vaccine, Live

Contagious Abortion (Strain 19) Vaccine, Live
Contagious Brucella Vaccine (Strain 19) Live

Brucella abortus (Strain 19 Vaccine) Vaccine, Live contains pure smooth culture of *Brucella abortus* strain 19 of low virulence in suitable diluent. The vaccine may be prepared immediately before use by reconstitution from the freeze-dried preparation with suitable diluent. The vaccine contains 4×10^{10} to 8×10^{10} *B. abortus* bacteria per dose.
This monograph applies to vaccines intended for the active immunisation of cattle and buffalo against Brucellosis caused by *B. abortus.*

Production

Preparation of vaccine. *B. abortus* may be grown as static culture on suitable solid medium or in suspension cultures by using bioreactors in suitable liquid medium. The bacterial cultures may be partially purified to reduce the components of spent media during manufacturing of bulk preparation (drug substance). The cultures are mixed with suitable stabilizing liquid before freeze drying, in case of freeze-dried vaccine preparations.

Choice of vaccine composition. *B. abortus* strain 19 having normal properties of a biovar 1 strain of *B. abortus,* but does not require CO_2 for growth, does not grow in presence of benzyl penicillin (5 IU per ml), thionin blue (20 µg per ml) and i-erythritol (1 mg per ml) concentrations. The strain displays minimal pathogenicity for guinea-pigs. *B. abortus* strain 19 seed should be procured from a reference laboratory and the master seed lot should be preserved by lyophilization or by freezing in liquid nitrogen.

Master seed lot

The seed lot vaccine strain should comply with the tests for purity, identity and smoothness characteristics of the organism and, a batch of vaccine prepared from the seed lot should comply with entire range of following control tests.

Identification. *B. abortus* present in the vaccine is identified by suitable bacterial morphology and microbial culture characteristics, along with suitable biochemical, molecular or serological assays. Gram's staining shows uniform pure short pink rods indicating a Gram-negative bacterium. Biochemical tests for oxidase and hydrogen sulphide production indicate the identity of bacteria. A suitable serological test such as agglutination test may be employed. A suitable molecular method such as polymerase chain reaction can be used to establish the identity of S19 strain of bacteria.

Safety. The S19 vaccines show reduced virulence, but should keep a minimal virulence to be efficient.
The master seed may be tested for safety in guinea pigs or cattle as follows.
Inject each of a group of at least 10 healthy adult guinea-pigs with the test vaccine diluted in buffered saline (pH 6.4), containing 5×10^{9} viable organisms or $1/10^{th}$ of calf dose intramuscularly and observe for 10 days.

The master seed lot passes the safety test if none of the animals shows notable adverse reactions or death attributable to vaccination.

Use 12 female calves of 4-6 months of age and six female calves are injected with double dose of vaccine prepared from the master seed lot by the route recommended by the manufacturer. Keep each lot of six calves separately and observe for 21 days. No significant local or systemic reaction should occur. If the test is performed satisfactorily with the vaccine prepared from a seed lot, this test does not have to be repeated routinely on vaccine lots prepared from the same seed lot.

Test for reversion to virulence. Prepare adequate suspensions of either the *B. abortus* S19 seed lot to be tested (test vaccine) and the S19 original seed culture (as a reference strain). For this, harvest 24 - 48 hours growth of each strain in sterile buffered saline solution (BSS: NaCl 8.5 g; KH_2PO_4 1.0 g; K_2HPO_4 2.0 g; purified water 1000 ml; pH 6.8 ± 0.2) and adjust the suspension in BSS to 10^9 CFU/ml using a spectrophotometer (0.170 OD when read at 600 nm). The exact number of CFU/ml should be checked afterwards by plating serial tenfold dilutions on to adequate culture medium (blood agar base or TSA are recommended). Inject subcutaneously 0.1 ml (10^8 CFU/mouse) of the suspension containing the test vaccine into each of 32 female CD1 mice, aged 5– 6 weeks. Carry out, in parallel, a similar inoculation in another 32 mice using the suspension containing the S19 reference strain. Euthanize the mice by cervical dislocation, in groups of eight selected at random 3, 6, 9 and 12 weeks later. Remove the spleens and homogenise individually and aseptically with a glass grinder (or in adequate sterile bags with a Stomacher) in 1 ml of sterile BSS. Spread each whole spleen suspension into onto several plates containing a suitable culture medium and incubate in standard *Brucella* conditions for 5-7 days (lower limit of detection: 1 bacterium per spleen). An animal is considered infected when at least 1 CFU is isolated from the spleen. Calculate the 50 per cent persistence time or 50 per cent recovery time (RT50) by the SAS® statistical method specifically developed for RT50 calculations. For this, determine the number of cured mice (no colonies isolated in the spleen) at each slaughtering point time (eight mice per point) and calculate the percentage of cured accumulated mice over time, by the Reed and Muench method. The function of distribution of this percentage describes a sigmoid curve, which must be linearised for calculating the RT50 values, using the computerised PROBIT procedure of the SAS® statistical package. Compare statistically the parallelism (intercept and slope) between the distribution lines obtained for both tested and reference S19 strains using the SAS® file specifically designed for this purpose. Two RT50 values can be statistically compared exclusively when they come from parallel distribution lines. If parallelism does not exist, the residual virulence of the tested strain should be considered inadequate, and discarded for vaccine production. If the parallelism is confirmed, compare statistically the RT50 values obtained for both tested and reference S19 strains using a SAS® file specifically designed for this purpose. To be accepted for vaccine production, the RT50 obtained with the tested strain should not differ significantly from that obtained with the reference S19 strain (RT50 and confidence limits are usually around 7.0 ± 1.3 weeks).

If this test has been performed with satisfactory results on a representative seed lot, it does not have to be repeated routinely on other seed lots and vaccine batches prepared from the same seed strain and using the same manufacturing process.

Immunogenicity.

A. Immunogenicity in guinea pigs. Inject each of a group of 10 healthy adult guinea-pigs drawn from a uniform stock and each weighing 300 g to 450 g, intramuscularly with $1/15^{th}$ of the calf dose of the test vaccine. Nine weeks later, challenge each of the vaccinated guinea pigs with 1 ml suspension of 5000 fully virulent *B. abortus* strain 544 organisms. Use 6 guinea-pigs of the same stock and weight as unvaccinated controls. Six weeks later, sacrifice all guinea-pigs and prepare cultures from their spleens. The master seed lot passes the test if not more than 25 per cent of the vaccinated animals contain demonstrable *B. abortus* organisms in spleens. The test is not valid unless the spleens of all control animals are infected.

B. *Immunogenicity in mice*. This test uses three groups of six female CD1 mice, aged 5–7 weeks that have been selected at random. 1) Prepare and adjust spectrophotometrically the vaccine suspensions as indicated above. 2) Inject subcutaneously a suspension containing 10^5 CFU (in a volume of 0.1 ml/mouse) of the vaccine to be examined (test vaccine) into each of six mice of the first group. 3) Inject subcutaneously a suspension containing 10^5 CFU of live bacteria of a reference S19 vaccine into each of six mice of the second group. The third group will serve as the unvaccinated control group and should be inoculated subcutaneously with 0.1 ml of BSS. 4) The exact number of CFU inoculated should be checked afterwards by plating serial ten-fold dilutions onto adequate culture medium (blood agar base or TSA are recommended). 5) All the mice are challenged 30 days after vaccination (and immediately following 16 hours starvation), intraperitoneally with a suspension (0.1 ml/mouse) containing 2×10^5 CFU of *B. abortus* strain 544 (CO_2 dependent), prepared, adjusted and retrospectively checked as above. 6) Kill the mice by cervical dislocation 15 days later. 7) Each spleen is excised aseptically, the fat is removed, and the spleen is weighed and homogenised. 8) Alternatively, the spleens can be frozen and kept at $\leq -16°$ for from 24 hours to 7 weeks. 9) Each spleen is homogenised aseptically with a glass grinder (or in adequate sterile bags in Stomacher) in nine times its weight of BSS, pH 6.8 ± 0.2 and three serial tenfold dilutions (1/10, 1/100 and 1/1000) of each homogenate made in the same diluent. Spread 0.2 ml of each dilution by quadruplicate in agar plates and incubate two of the plates in a 10 per cent CO_2 atmosphere (allows the growth of both vaccine and challenge strains) and the other two plates in air (inhibits the growth of the *B. abortus* 544 CO_2 dependent challenge strain), both at 37° ± 2° for 5 days. 10) Colonies of Brucella (*B. abortus* 544) should be enumerated on the dilutions corresponding to plates showing fewer than 300 CFU. When no colony is seen in the plates corresponding to the 1/10 dilution, the spleen is considered to be infected with five bacteria. These numbers of Brucella per spleen are first recorded as X and expressed as Y, after the following transformation: Y = log (X/log X). Mean and standard deviation, which are the response of each group of six mice, are then calculated. 11) The conditions of the control experiment are satisfactory when: i) the response of unvaccinated mice (mean of Y) is at least of 4.5; ii) the response of mice vaccinated with the reference S19 vaccine is lower than 2.5; and iii) the standard deviation calculated on each lot of six mice is lower than 0.8. 12) Carry out the statistical comparisons (the least significant differences test is recommended) of the immunogenicity values obtained in mice vaccinated with the S19 strain to be tested with respect to those obtained in mice vaccinated with the reference vaccine and in the unvaccinated control group. The test vaccine would be satisfactory if the immunogenicity value obtained in mice vaccinated with this vaccine is significantly lower than that

obtained in the unvaccinated controls and, moreover, does not differ significantly from that obtained in mice vaccinated with the reference vaccine.

Manufacturer's test

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 test for sterility except for the presence of vaccine micro-organism. Carry out the test by microscopic examination and by inoculation of suitable growth media. The bulk does not contain contaminating bacteria and fungi.

Enumeration of live Bacteria and determination of smoothness. Conduct viable count on final bulk of organisms by plate count method using a suitable medium. The viable bacterial count in the bulk preparations shall be much higher than that of the final formulated vaccine and a suitable in-house specification can be developed by the manufacturer. At least 90 per cent of the organisms should be in smooth phase.

Batch Test

Description. The vaccine may be in liquid form, off white in colour, is turbid containing bacterial cell suspension. If freeze-dried, the vaccine appears as off-white cake and up on its reconstitution, the attributes of liquid form may appear.

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

Viable count and smoothness. The vaccine complies with the requirements of the test, if the number of live *B. abortus* organisms is not less than 4×10^{10} to 8×10^{10} per dose stated on label and at least 90 per cent of the organisms are in smooth phase.

Bacterial and fungal contamination (2.2.11). The vaccine complies with the test for sterility except for the presence of vaccine micro-organism.

Safety. The vaccine complies with the safety tests 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 tests mentioned under section of master seed lot. If the batch has been prepared through the same manufacturing process from the same seed lot which has shown satisfactory results for potency, it is not necessary to conduct the test on each batch of vaccine.

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; (4) liquid preparation should not be allowed to freeze; (5) the vaccine is intended for use only in female calves of 4 to 8 months old; (6) storage temperatures; (7) The vaccine is not to be used in pregnant animals, specify stage of pregnancy; (8) Total volume and number of doses.