Edition: BP 2025 (Ph. Eur. 11.6 update)

Brucella Melitensis (Strain Rev. 1) Vaccine, Living

General Notices

(Brucellosis Vaccine (Live) (Brucella Melitensis Rev. 1 Strain) for Veterinary Use, Ph. Eur. monograph 0793)

Ph Eur

1 DEFINITION

Brucellosis vaccine (live) (Brucella melitensis Rev. 1 strain) for veterinary use is a suspension of live *Brucella melitensis* Rev. 1 strain. The vaccine contains not fewer than 0.5×10^9 and not more than 4×10^9 live bacteria per dose. This monograph applies to vaccines intended for the active immunisation of sheep and goats against disease caused by *B. melitensis*.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

B. melitensis Rev. 1 strain is cultured in a suitable medium. The method of culture is such as to avoid bacterial dissociation and thus maintain the smooth characteristic of the culture. The bacteria are suspended in a buffer solution that may contain a suitable stabiliser. The suspension is distributed into containers.

2-2 CHOICE OF VACCINE STRAIN

The vaccine strain is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the sheep and goats for which it is intended.

The following tests for safety (section 2-2-1), residual virulence (section 2-2-2), determination of dissociation phase of master seed lot (section 2-2-3) and immunogenicity in mice (section 2-2-4) may be used during the demonstration of safety and efficacy.

2-2-1 Safety

Use 8 sheep, 4-6 months old, that do not have antibodies against *B. melitensis*. Administer to each sheep by a route to be recommended 3 doses of the vaccine. Observe the sheep at least daily for at least 14 days.

The vaccine complies with the test if no sheep shows notable signs of disease or dies from causes attributable to the vaccine.

2-2-2 Residual virulence

The test is carried out on the master seed lot and on a representative batch of vaccine. If the quantity of the master seed sufficient for performing the test is not available, the lowest passage seed used for the production that is available in sufficient quantity may be used.

Use 32 female CD1 mice, 5-6 weeks old. Vaccinate each mouse by the subcutaneous route with a suspension (0.1 mL) containing 10^8 live bacteria. Euthanise the mice in groups of 8, selected at random, 3, 6, 9 and 12 weeks later. Remove the spleens and homogenise individually and aseptically in 1 mL of phosphate buffered saline pH 6.8 R. Spread the entire suspension on plates containing a suitable culture medium (lower limit of detection: 1 bacterium per spleen). Carry out in parallel a similar test using a suitable reference strain of *Brucella melitensis* Rev. 1. Calculate the 50 per cent persistence time by the usual statistical methods (*5.3*) for probit analysis.

The product complies with the test if the 50 per cent persistence time for the vaccine strain does not differ significantly from that of the reference strain.

2-2-3 Determination of dissociation phase of the master seed lot

Examine not fewer than 200 colonies by a suitable technique. The culture of the vaccine strain is seen to be in the smooth (S) phase.

The seed lot complies with the test if not fewer than 99 per cent of the colonies are of the smooth type.

2-2-4 Immunogenicity in mice

The test is carried out on the master seed lot and on a representative batch of vaccine. If the quantity of the master seed sufficient for performing the test is not available, the lowest passage seed used for the production that is available in sufficient quantity may be used.

Use for the test healthy CD1 female mice, 5-7 weeks old and from the same stock. Distribute the mice into 3 groups of 6 mice. Dilute the vaccine strain and a suitable reference strain of *Brucella melitensis* Rev. 1 to a concentration of 10^6 CFU/mL.

Vaccinate by the subcutaneous route the mice of the 1^{st} group with 0.1 mL of the diluted vaccine strain and the mice of the 2^{nd} group with 0.1 mL of the diluted reference strain; keep the 3^{rd} group as the unvaccinated control. After 30 days, challenge all the mice with 2×10^5 bacteria of *B. abortus* strain 544 (CO_2-dependent). Euthanise the mice 15 days later and remove the spleen for *B. abortus* isolation. Record the number of *B. abortus* per spleen (X) and transform this value to obtain $Y = \log_{10} (X/\log_{10} X)$. Then calculate the mean and standard deviation of each group.

The test is valid if:

— the mean of the unvaccinated control group is at least 4.5 (mean of Y);

— the mean of the group receiving the reference strain is lower than 2.5 (mean of Y); and

— the standard deviation of each group is lower than 0.8.

Carry out a statistical comparison of the immunogenicity values of the 3 groups using the least significant differences test. The vaccine strain complies with the test if:

— the immunogenicity value of the group receiving the vaccine strain is significantly lower than the immunogenicity value of the control group; and

— the immunogenicity value of the group receiving the vaccine strain is not significantly different from the immunogenicity value of the group receiving the reference strain.

3 BATCH TESTS

3-1 Identification

B. melitensis present in the vaccine is identified by suitable morphological, serological and biochemical tests and by culture: Rev. 1 strain is inhibited by addition to the suitable culture medium of either benzylpenicillin sodium (3 µg/mL), thionin (20 µg/mL) or basic fuchsin (20 µg/mL); the strain grows on agar containing 2.5 µg of streptomycin per millilitre.

3-2 Determination of dissociation phase

Examine not fewer than 200 colonies by a suitable technique. The culture of the vaccine strain is seen to be in the smooth (S) phase.

The vaccine complies with the test if not fewer than 95 per cent of the colonies are of the smooth type.

3-3 Bacteria and fungi

The vaccine complies with the test if it does not contain extraneous micro-organisms. Verify the absence of micro-organisms other than *B. melitensis* Rev. 1 strain as described in the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-4 Live bacteria

Make a count of live bacteria on a solid medium suitable for the culture of *B. melitensis* Rev. 1 strain.

The vaccine complies with the test if it contains not fewer than 0.5×10^9 and not more than 4×10^9 live bacteria per dose.

4 LABELLING

The label states:

— that the vaccine may be dangerous for man;

— that the vaccine is not to be used in pregnant animals;

— that the vaccine may be dangerous for cattle and that they are not to be kept in contact with sheep or goats vaccinated less than 24 h previously.