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

Rabies Veterinary Vaccine, Inactivated

General Notices

(Rabies Vaccine (Inactivated) for Veterinary Use, Ph. Eur. monograph 0451)

Ph Eur

1 DEFINITION

Rabies vaccine (inactivated) for veterinary use is a preparation of a suitable strain of fixed rabies virus, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of animals against rabies.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine is prepared from virus grown either in suitable cell lines or in primary cell cultures from healthy animals (*5.2.4*). The virus suspension is harvested on one or more occasions within 28 days of inoculation. Multiple harvests from a single production cell culture may be pooled and considered as a single harvest.

The virus harvest is inactivated. The vaccine may be adjuvanted.

2-2 SUBSTRATE FOR VIRUS PROPAGATION

2-2-1 Cell cultures

The cell cultures comply with the requirements for cell cultures for the production of veterinary vaccines (*5.2.4*).

2-3 CHOICE OF VACCINE COMPOSITION

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

The following tests for safety (section 2-3-1) and immunogenicity (section 2-3-2) may be used during the demonstration of safety and efficacy in cats and dogs.

The suitability of the vaccine with respect to immunogenicity (section 2-3-2) for carnivores (cats and dogs) is demonstrated by direct challenge. For other species, if a challenge test has been carried out for the vaccine in cats or dogs, an indirect test is carried out by determining the antibody level following vaccination of not fewer than 20 animals according to the schedule to be recommended; the vaccine is satisfactory if, after the period to be claimed for protection, the mean rabies virus antibody level in the serum of the animals is not less than 0.5 IU/mL and if not more than 10 per cent of the animals have an antibody level less than 0.1 IU/mL.

2-3-1 Safety

Carry out the test for each route and method of administration to be recommended for vaccination. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test, use not fewer than 8 animals of the minimum age to be recommended and that do not have antibodies against rabies virus. Administer to each animal 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer 1 dose after an interval of at least 14 days. Observe the animals at least daily for at least 14 days after the last administration.

The vaccine complies with the test if no animal shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-3-2 Immunogenicity

Each test is carried out for each route and method of administration to be recommended, using in each case animals of the minimum age to be recommended for vaccination. The vaccine administered to each animal is of minimum potency.

Use for the test not fewer than 35 animals. Take a blood sample from each animal and test individually for antibodies against rabies virus to determine susceptibility. Vaccinate not fewer than 25 animals, according to the schedule to be recommended. Maintain not fewer than 10 animals as controls. Observe all the animals for a period equal to the claimed duration of immunity. No animal shows signs of rabies. On the last day of the claimed period for duration of immunity or later, challenge each animal by intramuscular injection with a sufficient quantity of virulent rabies virus of a strain approved by the competent authority. Observe the animals at least daily for 90 days after challenge. Animals that die from causes not attributable to rabies are eliminated. The test is not valid if the number of such deaths reduces the number of vaccinated animals in the test to fewer than 25 and the test is invalid unless at least 8 control animals (or a statistically equivalent number if more than 10 control animals are challenged) show signs of rabies and the presence of rabies virus in their brain is demonstrated by the fluorescent-antibody test or some other suitable method. The vaccine complies with the test if not more than 2 of the 25 vaccinated animals (or a statistically equivalent number if more than 25 vaccinated animals are challenged) show signs of rabies.

2-4 MANUFACTURER'S TESTS

2-4-1 Residual live virus

The test for residual live virus is carried out by inoculation of the inactivated virus into the same type of cell culture as that used in the production of the vaccine or a cell culture shown to be at least as sensitive. The quantity of inactivated virus harvest used is equivalent to not less than 25 doses of the vaccine. After incubation for 4 days, a subculture is made using trypsinised cells; after incubation for a further 4 days, the cultures are examined for residual live rabies virus by an immunofluorescence test. The inactivated virus harvest complies with the test if no live virus is detected.

2-4-2 Antigen content of the harvest

The content of rabies virus glycoprotein is determined by a suitable immunochemical method (*2.7.1*). The content is within the limits approved for the particular preparation.

2-4-3 Antigen content of the pooled harvest

The quantity of rabies virus glycoprotein per dose, determined by a suitable immunochemical method (*2.7.1*) on the pooled harvest immediately before blending, is not significantly lower than that of a batch of vaccine that gave satisfactory results in the test described under Potency.

2-4-4 Batch potency test

It is not necessary to carry out the potency test (section 3-4) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. An alternative validated method such as an immunosorbent assay is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, such an alternative validated method must be used for

routine testing. The following serological assay has been shown to be suitable[1] and may be used provided the test for antigen content of the pooled harvest (section 2-4-3) has been carried out with satisfactory results.

Use groups of not fewer than 8 female mice (strain NMRI), each weighing 18-20 g. Prepare a 1 IU/mL suspension of *rabies vaccine (inactivated) for veterinary use BRP* using phosphate-buffered saline (PBS) for dilution. Vaccines with a minimum potency requirement of 1 IU/mL are used without further dilution. Vaccines with a minimum potency requirement of more than 1 IU/mL are diluted with PBS to contain approximately, but not less than, 1 IU/mL. Administer by the intraperitoneal route to each mouse of one group 0.2 mL of the vaccine, diluted where necessary, and to each mouse of another group 0.2 mL of the suspension of *rabies vaccine (inactivated) for veterinary use BRP*. Take blood samples 14 days after the injection and test the sera individually for rabies antibody using a suitable virus neutralisation test, for example the rapid fluorescent focus inhibition test (RFFIT) described for *Human rabies immunoglobulin (0723)* or a suitable validated modification of the RFFIT[2].

The test is not valid if more than 2 mice injected with the suspension of *rabies vaccine (inactivated) for veterinary use BRP* show no antibodies in their serum.

Individual serum titres are determined with an appropriate anti-rabies immunoglobulin reference.

The antibody titre of mice receiving the suspension of *rabies vaccine (inactivated) for veterinary use BRP* is compared to the antibody titre of mice receiving the vaccine using a suitable statistical approach (*5.3*).

The vaccine complies with the test if the antibody titre of mice injected with the vaccine is significantly higher than that of mice injected with the suspension of *rabies vaccine (inactivated) for veterinary use BRP*.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Residual live virus

This test may be omitted for batch release, as stated in the monograph Vaccines for veterinary use (0062).

Carry out a suitable amplification test for residual live virus using a pool of the contents of 5 containers by inoculating a cell culture of the same type as that used in the production of the vaccine or a cell culture shown to be at least as sensitive. The vaccine complies with the test if no live virus is detected.

If the vaccine contains an adjuvant, separate the adjuvant from the liquid phase, by a method that does not inactivate the virus or otherwise interfere with the detection of live viruses, or carry out a test for inactivation on the mixture of bulk antigens before addition of the adjuvant.

3-4 Potency

The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the clinical effects of the dose of rabies virus defined below, administered intracerebrally, with the quantity of a reference preparation, calibrated in International Units, necessary to provide the same protection.

The International Unit is the activity of a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Rabies vaccine (inactivated) for veterinary use BRP is calibrated in International Units against the International Standard.

The test described below uses a parallel-line model with at least 3 points for the vaccine to be examined and the reference preparation. Once the analyst has experience with the method for a given vaccine, it is possible to carry out a simplified test using 1 dilution of the vaccine to be examined. Such a test enables the analyst to determine that the vaccine has a potency significantly higher than the required minimum but will not give full information on the validity of each individual potency determination. It allows a considerable reduction in the number of animals required for the test and should be

considered by each laboratory in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Selection and distribution of the test animals Use in the test healthy female mice about 4 weeks old and from the same stock. Distribute the mice into at least 10 groups of not fewer than 10 mice.

Preparation of the challenge suspension Inoculate a group of mice intracerebrally with the Challenge Virus Standard (CVS) strain of rabies virus and when the mice show signs of rabies, but before they die, euthanise the mice and remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant as challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below -60 °C. Thaw 1 ampoule of the suspension and make serial dilutions in a suitable diluent. Allocate each dilution to a group of mice and inject intracerebrally into each mouse 0.03 mL of the dilution allocated to its group. Observe the animals at least daily for 14 days and record the number in each group that, between the 5^{th} and the 14^{th} days, develop signs of rabies. Calculate the ID_{50} of the undiluted suspension.

Determination of potency of the vaccine to be examined Prepare at least 3 serial dilutions of the vaccine to be examined and 3 similar dilutions of the reference preparation. Prepare the dilutions such that those containing the largest quantity of vaccine may be expected to protect more than 50 per cent of the animals into which they are injected and those containing the smallest quantities of vaccine may be expected to protect less than 50 per cent of the animals into which they are injected. Allocate each dilution to a different group of mice and inject by the intraperitoneal route into each mouse 0.5 mL of the dilution allocated to its group. 14 days after the injection prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, it contains about 50 ID_{50} in each 0.03 mL. Inject intracerebrally into each vaccinated mouse 0.03 mL of this suspension. Prepare 3 suitable serial dilutions of the challenge suspension. Allocate the challenge suspension and the 3 dilutions one to each of 4 groups of 10 unvaccinated mice and inject intracerebrally into each mouse 0.03 mL of the suspension or one of the dilutions allocated to its group. Observe the animals in each group at least daily for 14 days. The test is not valid if more than 2 mice of any group die within the first 4 days after challenge. Record the numbers in each group that show signs of rabies in the period 5 days to 14 days after challenge.

The test is not valid unless:

— for both the vaccine to be examined and the reference preparation, the 50 per cent protective dose lies between the smallest and the largest dose given to the mice;

— the titration of the challenge suspension shows that 0.03 mL of the suspension contained at least 10 ID_{50};

— the confidence limits (P = 0.95) are not less than 25 per cent and not more than 400 per cent of the estimated potency; when this validity criteria is not met, the lower limit of the estimated potency must be at least 1 IU in the smallest prescribed dose;

— the statistical analysis shows a significant slope (P = 0.95) and no significant deviations from linearity or parallelism of the dose-response lines (P = 0.99).

The vaccine complies with the test if the estimated potency is not less than 1 IU in the smallest prescribed dose.

Application of alternative end-points Once a laboratory has established the above assay for routine use, the lethal end-point is replaced by an observation of clinical signs and application of an end-point earlier than death to reduce animal suffering. The following is given as an example.

The progress of rabies infection in mice following intracerebral injection can be represented by 5 stages defined by typical clinical signs:

Stage 1: ruffled fur, hunched back;
Stage 2: slow movements, loss of alertness (circular movements may also occur);
Stage 3: shaky movements, trembling, convulsions;
Stage 4: signs of paresis or paralysis;
Stage 5: moribund state.

Mice are observed at least twice daily from day 4 after challenge. Clinical signs are recorded using a chart such as that shown in Table 0451.-1. Experience has shown that using stage 3 as an end-point yields assay results equivalent to those found when a lethal end-point is used. This must be verified by each laboratory by scoring a suitable number of assays using both clinical signs and the lethal end-point.

Table 0451.-1. – *Example of a chart used to record clinical signs in the rabies vaccine potency test*

	Days after challenge							
Clinical signs	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Ruffled fur								

	Days after challenge							
Clinical signs	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Hunched back								
Slow movements Loss of alertness Circular movements								
Shaky movements Trembling Convulsions								
Paresis Paralysis								
Moribund state								

4 LABELLING

The label states:

— the type of cell culture used to prepare the vaccine and the species of origin;

— the minimum number of International Units per dose;

— the minimum period for which the vaccine provides protection.

Ph Eur

[1] B. Krämer *et al*. Collaborative Study for Validation of a Serological Potency Assay for Rabies Vaccines (inactivated) for Veterinary Use, *Pharmeur Bio Sci Notes* 2010(2):37-55.

[2] B. Krämer *et al*. The rapid fluorescent focus inhibition test is a suitable method for batch potency testing of inactivated rabies vaccine. *Biologicals* 2009;37:119-126.