Rabies vaccine (inactivated) for veterinary use

EUROPEAN PHARMACOPOEIA 5.0

Vaccinum rabiei inactivatum

ad usum veterinarium

DEFINITION

Rabies vaccine (inactivated) for veterinary use is a liquid or freeze-dried preparation of fixed rabies virus inactivated by a suitable method.

PRODUCTION

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 rabies virus is inactivated by a suitable method.

Inactivation. The test for residual live rabies 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 used in the test 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. No live virus is detected.

Antigen content. 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.

The vaccine may contain one or more adjuvants.

CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to immunogenicity for each species for which it is recommended. The suitability of the vaccine with respect to immunogenicity 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 twenty animals according to the recommended schedule; the vaccine is satisfactory if, after the period 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. The test described below may be used to demonstrate immunogenicity in cats and dogs.

Immunogenicity. Use not fewer than 35 susceptible animals of the minimum age recommended for vaccination. Take a blood sample from each animal and test individually for antibodies against rabies virus to determine susceptibility. Administer by the recommended route to each of not fewer than 25 animals one dose of vaccine. Keep 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 all animals by intramuscular injection of virulent rabies virus of a strain approved by the competent authority. Observe the animals for 90 days. 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. The test is not valid unless at least eight 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.

LABELLING

The label states the protective antigens present in the vaccine.

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RABIES VACCINE (INACTIVATED) FOR VETERINARY USE

Vaccine and Active principles

The vaccine is a suspension of inactivated rabies virus, prepared by suitable methods from cell cultures infected with rabies virus. It contains the protective antigens of the rabies virus, derived from a toxigenic strain. The vaccine is shown to be satisfactory with respect to immunogenicity and to be safe for veterinary use.

Stability

The vaccine is stable at temperatures up to 40°C in its amber glass vials. It is not stable in sterile water for injection, as the antigens are denatured. The vaccine should be protected from light when it is in use.

Inactivation

The virus is inactivated by a suitable method. The vaccine complies with the test for residual live virus if no live virus is detected after incubation of cultures inoculated with the vaccine for an additional 4 days. It complies with the test for a toxigenic strain of P. multocida if a culture of P. multocida is grown in the vaccine and shows signs of rabies.

LABELLING

The label states the protective antigens present in the vaccine.
fluorescent focus inhibition test described for Human rabies immunoglobulin (0723). The amount of antibody is not less than that produced by a vaccine that has been found satisfactory with respect to immunogenicity as described above or in the test described under Potency.

**Antigen content.** The quantity of rabies virus glycoprotein per dose, determined by a suitable immunochemical method (2.7.3), is not significantly lower than that of a batch that has been found satisfactory with respect to immunogenicity as described above or in the test described under Potency.

**IDENTIFICATION**

When injected into animals, the vaccine stimulates the production of specific neutralising antibodies.

**TESTS**

**Safety.** If the vaccine is intended for more than one species including one belonging to the order of Carnivora, carry out the test in dogs. Otherwise use one of the species for which the vaccine is intended. Administer, by a recommended route, a double dose of vaccine to each of 2 animals having no antibodies against rabies virus. Observe the animals for 14 days. No abnormal local or systemic reaction occurs.

**Inactivation.** Carry out the test using a pool of the contents of 5 containers.

For vaccines which do not contain an adjuvant, carry out a suitable amplification test for residual infectious rabies virus using 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. No live virus is detected.

For vaccines that contain an adjuvant, inject intracerebrally into each of not fewer than 10 mice each weighing 11 g to 15 g 0.03 ml of a pool of at least 5 times the smallest stated dose. To avoid interference from any antimicrobial preservative or the adjuvant, the vaccine may be diluted not more than 10 times before injection. In this case or if the vaccine strain is pathogenic only for unweaned mice, carry out the test on mice 1 to 4 days old. Observe the animals for 21 days. If more than 2 animals die during the first 48 h, repeat the test. From the third to the twenty-first days following the injection, the animals show no signs of rabies and immunofluorescence tests carried out on the brains of the animals show no indication of the presence of rabies virus.

**Sterility.** The vaccine complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

**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 Organisation.

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 one 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 CVS strain of rabies virus and when the mice show signs of rabies, but before they die, kill 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 liquid as challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below –60 °C. Thaw one 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 for 14 days and record the number in each group that, between the fifth and the fourteenth 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 three serial dilutions of the vaccine to be examined and three 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 intracerebrally 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 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,

- the statistical analysis shows a significant slope and no significant deviations from linearity or parallelism of the dose-response lines.
The vaccine complies with the test if the estimated potency is not less than 1 IU in the smallest prescribed dose.

**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.

**RABIES VACCINE (LIVE, ORAL) FOR FOXES**

Vaccinum rabiei perorale vivum ad vulpem

**DEFINITION**

Rabies vaccine (live, oral) for foxes is a preparation of an immunogenic strain of an attenuated rabies virus. The vaccine is incorporated in a bait in such a manner as to enable the tests prescribed below to be performed aseptically.

**PRODUCTION**

The attenuated virus strain is grown in suitable cell cultures (5.2.4); if the cell cultures are of mammalian origin, they are shown to be free from rabies virus. The virus suspension is harvested on one or more occasions within 14 days of inoculation. Multiple harvests from a single cell lot may be pooled and considered as a single harvest. The viral suspension may be mixed with a suitable stabiliser. The vaccine may be freeze-dried or liquid. A freeze-dried vaccine has to be reconstituted before use.

**CHOICE OF VACCINE STRAIN**

Only a virus strain shown to be satisfactory with respect to immunogenicity (see Potency) and the following characteristics may be used in the preparation of the vaccine:

– when administered orally at the dose and by the method recommended for use to forty foxes, it causes no sign of rabies within 180 days of administration,
– when administered orally at ten times the recommended dose to each of ten foxes, it causes no sign of rabies within 180 days of administration,
– when administered orally at ten times the recommended dose to each of ten dogs, it causes no sign of rabies within 180 days of administration,
– when administered orally at ten times the recommended dose to each of ten cats, it causes no sign of rabies within 180 days of administration,
– in natural and experimental conditions, the virus strain does not spread from one animal to another in wild rodents,
– the virus strain has one or more stable genetic markers that may be used to discriminate the vaccine strain from other rabies virus strains.

**BATCH TESTING**

If the test for potency has been carried out with satisfactory results on a representative batch of vaccine, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot.

**IDENTIFICATION**

A. When mixed with a monospecific rabies antiserum, the vaccine is no longer able to infect susceptible cell cultures into which it is inoculated.

B. A test is carried out to demonstrate the presence of the genetic marker.

**TESTS**

**Extraneous viruses**

(a) Mix the vaccine with a specific neutralising rabies virus antiserum. It no longer provokes cytopathic effects in susceptible cell cultures. It shows no evidence of haemagglutinating or haemadsorbing agents.

(b) Inoculate 1 in 10 and 1 in 1000 dilutions of the vaccine into susceptible cell cultures. Incubate at 37 °C. After 2, 4 and 6 days, stain the cells with a panel of monoclonal antibodies that do not react with the vaccine strain but that react with other strains of rabies virus (for example, street virus, Pasteur strain). The vaccine shows no evidence of contaminating rabies virus.

**Bacterial and fungal contamination.** The vaccine complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

**Mycoplasmas (2.6.7).** The vaccine complies with the test for mycoplasmas.

**Virus titre.** Titrate the vaccine in suitable cell cultures. One dose of the vaccine contains not less than the quantity of virus equivalent to the minimum titre stated on the label.

**POTENCY**

Use not fewer than thirty-five foxes, at least three months old, free from rabies-neutralising antibodies. Administer orally and with the bait stated on the label to each of not fewer than twenty-five animals a volume of the vaccine containing a quantity of virus equivalent to the minimum titre stated on the label. Keep not fewer than ten animals as controls. Observe all the animals for 180 days. No animal shows signs of rabies. The test is not valid if fewer than twenty-five vaccinated animals survive after this observation period. On the 180th day after vaccination, challenge all foxes by the intramuscular injection of virulent rabies virus of a strain approved by the competent authority. Observe the animals for 90 days. 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 twenty-five. The vaccine complies with the test if not more than two of twenty-five vaccinated animals (or a statistically equivalent number if more than twenty-five vaccinated animals are challenged) show signs of rabies. The test is not valid unless at least nine control animals (or a statistically equivalent number if more than ten 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 reliable method.

**LABELLING**

The label states the nature of the genetic marker of the virus strain.