Detection of cytopathic viruses. Two monolayers of at least 6 cm² each are stained with an appropriate cytological stain. Examine the entire area of each stained monolayer for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality that might be attributable to a contaminant.

Detection of haemadsorbent viruses. Monolayers totalling at least 70 cm² are washed several times with a suitable buffer solution and a sufficient volume of a suspension of suitable red blood cells added to cover the surface of the monolayer evenly. After different incubation times, examine cells for the presence of haemadsorption.

Detection of specified viruses. Tests are be carried out for freedom of contaminants specific for the species of origin of the cells and for the species for which the product is intended.

Tests in other cell cultures. Monolayers totalling at least 140 cm² are required. The cells are frozen and thawed at least three times and then centrifuged to remove cellular debris. Aliquots are inoculated onto the following cells at any time up to 70 per cent confluency:
- primary cells of the source species;
- cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
- cells sensitive to pestiviruses.

The inoculated cells are maintained in culture for at least 7 days, after which freeze-thawed extracts are prepared as above, and inoculated onto sufficient fresh cultures of the same cell types to allow for the testing as described below. The cells are cultured for at least a further 7 days. All cultures are regularly examined for the presence of any cytopathic changes indicative of living organisms.

At the end of this period of 14 days, the inoculated cells are subjected to the following checks:
- freedom from cytopathic and haemadsorbent organisms is demonstrated using the methods specified in the relevant paragraphs above;
- relevant substrates are tested for the absence of pestiviruses and other specific contaminants by immunofluorescence or other validated methods as indicated in the paragraph above on Detection of Specified Viruses.

In addition to the restrictions described below, manufacturers must consider restrictions on the handling of substances of animal origin in the vaccine manufacturing premises.

The restrictions imposed by these sections may need to be varied in accordance with changes in the incidence of disease in the country of origin and in Europe.

REQUIREMENTS

Substances of animal origin comply with the requirements of the Pharmacopoeia (where a relevant monograph exists).

Source. The risk related to the animal diseases occurring in the country of origin of the substance and to the potential infectious diseases occurring in the source species, in relation to the proposed recipient species must be carefully evaluated. The strictest possible selection criteria must be applied, in particular for substances for use in products intended for the same species and for substances of bovine, caprine, ovine and porcine origin.

Preparation. Substances of animal origin are prepared from a homogeneous bulk designated with a batch number. A batch may contain substances derived from as many animals as desired but once defined and given a batch number, the batch is not added to or contaminated in any way.

All batches of substances shall be shown to be free from contaminants as described below and/or subject to a validated inactivation procedure.

Inactivation. The inactivation procedure chosen shall have been shown to be capable of reducing the titre of certain potential contaminants in the substance to the level of contamination by at least 10⁶. If this reduction in titre cannot be shown experimentally, kinetic studies for the inactivation procedure must be carried out and shown to be satisfactory, taking into account the possible level of contamination.

The list of potential contaminating organisms that the procedure must be shown to be capable of inactivating must be appropriate to the particular species of origin of the substance. The evidence for the efficacy of the procedure, which must relate to the current circumstances, may take the form of references to published literature or experimental data generated by the manufacturer.

Tests. For examination of the substance for freedom from contaminants, any solids are dissolved or suspended in a suitable medium in such a way as to create a solution or suspension containing at least 300 g/l of the substance to be examined. If the substance is not soluble or where cytotoxic reactions occur, a lower concentration may be used.

Any batch of substance found to contain living organisms of any kind is unsatisfactory and is either discarded or repro-cessed and shown to be satisfactory.

Freedom from extraneous viruses. The solution or suspension of the solid substance or the undiluted liquid substance is tested for contaminants by suitably sensitive methods. These methods shall include tests in suitably validated method. Where the use of such substances has been shown to be essential and sterilisation is not possible, the criteria described under Requirements apply.

- Substances of animal origin used during production are either subjected to a suitable, validated sterilisation or inactivation procedure or the substance is tested for the absence of extraneous organisms in accordance with the Requirements below. For inactivated vaccines, the method used for inactivation of the vaccine strain may also be validated for inactivation of possible contaminants from substances of animal origin.
5.2.6. Evaluation of safety of veterinary vaccines

During the development of the vaccine, safety tests are carried out in the target species to show the risks from use of the vaccine. Live vaccines are prepared only from strains of organisms that have been shown to be safe.

In the tests, “dose” means that quantity of the product to be recommended for use and containing the maximum titre or potency likely to be contained in production batches. For live vaccines, a batch or batches of vaccine prepared from the least attenuated passage to be used for production shall be used in the tests.

The safety of each of the components of combined vaccines and the safety of the combined product shall be demonstrated. For inactivated vaccines, safety tests carried out on the combined vaccine may be regarded as sufficient to demonstrate the safety of the individual components.

The tests described below, modified or supplemented by tests described in the Production section of a specific monograph may be carried out as part of the tests necessary during development to demonstrate the safety of a vaccine.

A. LABORATORY TESTS

Safety of the administration of one dose. For each of the recommended routes of administration, administer one dose of vaccine to susceptible animals of each species and category for which use of the vaccine is to be recommended. This must include animals of the youngest recommended age and pregnant animals, if appropriate. The animals are observed and examined for signs of abnormal local and systemic reactions. Where appropriate, these studies shall include detailed post-mortem macroscopic and microscopic examinations of the injection site; such examinations are not usually necessary for non-food animals. Other objective criteria are recorded, such as rectal temperature (for mammals) and performance measurements. The rectal temperatures are recorded on at least the day before vaccination, at the time of vaccination, 4 h after vaccination and on the following 4 days. The animals are observed and examined until reactions may no longer be expected but, in all cases, the observation and examination period extends at least until 14 days after administration.

As part of these studies, examination of reproductive performance must also be considered when data suggest that the starting material from which the product is derived may be a risk factor. Where prescribed in a monograph, reproductive performance of males and non-pregnant and pregnant females and harmful effects on the progeny, including teratogenic and abortifacient effects, are investigated.

Safety of one administration of an overdose. An overdose of the product is administered by each recommended route of administration to animals of the most sensitive categories of the target species, including animals of the youngest age and pregnant animals, if appropriate. The overdose normally consists of 10 doses of a live vaccine or 2 doses of an inactivated product. The animals are observed and examined for signs of local and systemic reactions. Other objective criteria are recorded, such as rectal temperature (for mammals) and performance measurements. The animals are observed and examined for at least 14 days after administration.

Safety of the repeated administration of one dose. Repeated administration of one dose may be required to reveal any adverse effects induced by such administration. These tests are carried out on the most sensitive categories of the target species, using the recommended route of administration. The animals are observed and examined for at least 14 days after the last administration for signs of systemic and local reactions. Other objective criteria are recorded, such as rectal temperature (for mammals) and performance measurements.

Residues. It is not normally necessary to undertake a study of residues. However, where adjuvants and/or preservatives are used in the manufacture of veterinary vaccines, consideration shall be given to the possibility of any residue remaining in the foodstuffs. If necessary, the effects of such residues are investigated. Moreover, in the case of live vaccines for well established zoonotic diseases, the determination of residual vaccine organisms at the injection site may be required, in addition to the studies of dissemination described below.

Adverse effects on immunological functions. Where the vaccine might adversely affect the immune response of the vaccinated animal or of its progeny, suitable tests on the immunological functions are carried out.

Special requirements for live vaccines. The following laboratory tests must also be carried out with live vaccines. a) Spread of the vaccine strain. Spread of the vaccine strain from vaccinated to unvaccinated target animals is investigated using the recommended route of administration most likely to result in spread. Moreover, it may be necessary to investigate the safety of spread to non-target species that could be highly susceptible to a live vaccine strain. An assessment must be made of how many animal-to-animal passages are likely to be sustainable under normal circumstances together with an assessment of the likely consequences.

b) Dissemination in vaccinated animal. Faeces, urine, milk, eggs, oral, nasal and other secretions shall be tested for the presence of the organism. Moreover, studies may be required of the dissemination of the vaccine strain in the body, with particular attention being paid to the predilection sites for replication of the organism. In the case of live vaccines for well-established zoonotic diseases for food-producing animals, these studies are obligatory.

c) Reversion to or increase in virulence. For attenuated vaccines, use material from the passage level that is least attenuated for the target species between the master seed lot and the final product. For other live vaccines, use material from the passage likely to have maximum virulence for the target species. The initial vaccination is carried out using the recommended route of administration most likely to lead to reversion to virulence. After this, not fewer than 5 further serial passages through animals of the target species are undertaken. Where this is not technically possible due to failure of the organism to replicate adequately, the test is repeated and as many passages as possible are carried out in