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Appendix XV K (Vet) 1. Evaluation of Safety of Veterinary Vaccines and Immunosera

(Ph. Eur. general texts 5.2.6)

The term ‘product’ means either a vaccine or an immunoserum throughout the text.

During development, safety tests are carried out in the target species to show the risks from use of the product.

Immune status for tests on vaccines The immune status of animals to be used for the safety test is specified in the specific monograph. For most monographs, 1 of the 3 following categories is specified:

1) the animals must be free from antibodies against the virus/bacterium/toxin etc. contained in the vaccine;
2) the animals are preferably free from antibodies against the virus/bacterium/toxin etc. contained in the vaccine, but animals with a low level of antibody may be used as long as the animals have not been vaccinated and the administration of the vaccine does not cause an anamnestic response;
3) the animals must not have been vaccinated against the disease that the vaccine is intended to prevent.

As a general rule, category 1 is specified for live vaccines. For other vaccines, category 2 is usually specified, but where most animals available for use in tests would comply with category 1, this may be specified for inactivated vaccines also. Category 3 is specified for some inactivated vaccines where determination of antibodies prior to testing is unnecessary or impractical. For poultry vaccines, as a general rule the use of specified-pathogen-free (SPF) birds is specified.

For avian vaccines, the safety test is generally carried out using SPF chickens (*5.2.2*), except that for vaccines not recommended for use in chickens it is carried out using birds of one of the species for which the vaccine is recommended, the birds being free from antibodies against the disease agent for which the vaccine is intended to provide protection.

Vaccines In laboratory 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. Live vaccines are prepared only from strains of organisms that have been shown to be safe. For live vaccines, use a batch or batches of vaccine containing virus/bacteria at the least attenuated passage level that will be present in a batch of vaccine.

For combined vaccines, the safety shall be demonstrated; for live components of combined vaccines, compliance with the special requirements for live vaccines stated below shall be demonstrated separately for each vaccine strain.

For inactivated vaccines, safety tests carried out on the combined vaccine may be regarded as sufficient to demonstrate the safety of the individual components.

Immunosera In the tests, ‘dose’ means the maximum quantity of the product to be recommended for use and containing the maximum potency and maximum total protein likely to be contained in production batches. In addition, if appropriate, the dose tested also contains maximum quantities of immunoglobulin or gammaglobulin.

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

1 LABORATORY TESTS

1-1 SAFETY OF THE ADMINISTRATION OF 1 DOSE

For each of the recommended routes of administration, administer 1 dose of product to animals of each species and category for which use of the product is to be recommended. This must include animals that are expected to be the most

sensitive, usually animals of the youngest recommended age, unless otherwise specified in a specific monograph, and pregnant animals, if appropriate.

For vaccines intended for use in mammals, in general 8 animals per group are used unless otherwise justified or specified in a specific monograph.

For fish vaccines administered by immersion, bathe the fish for twice the recommended time using a bath at twice the recommended concentration.

For vaccines intended for use in fish, in general 50 fish per group are used unless otherwise justified or specified in a specific monograph.

For vaccines intended for use in birds older than 3 weeks, in general 8 birds per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in birds younger than 3 weeks, in general 10 birds per group are used unless otherwise justified or specified in a specific monograph.

The animals are observed and examined at least daily 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. Other objective criteria are recorded, such as body temperature (for mammals) and performance measurements. The body temperatures are recorded on at least the day before and at the time of administration of the product, 4 h later and on the following 4 days. The animals are observed and examined at least daily until reactions may no longer be expected but, in all cases, the observation and examination period extends at least until 14 days after administration.

Unless otherwise prescribed in a specific monograph or, in the absence of a specific monograph, unless otherwise justified and authorised, the vaccine complies with the test if no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the vaccine.

1-2 SAFETY OF 1 ADMINISTRATION OF AN OVERDOSE

Overdose testing is required only for live vaccines. An overdose of the product is administered by each recommended route of administration to animals of the categories of the target species that are expected to be the most sensitive, such as animals of the youngest age. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If 1 route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. The overdose normally consists of 10 doses of a live vaccine. For freeze-dried live vaccines, the 10 doses shall be reconstituted in a suitable volume of diluent for the test. For vaccines intended for use in mammals, in general 8 animals per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in fish, in general 50 fish per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in birds older than 3 weeks, in general 8 birds per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in birds younger than 3 weeks, in general 10 birds per group are used unless otherwise justified or specified in a specific monograph. The animals are observed and examined at least daily for signs of local and systemic reactions. Other objective criteria are recorded, such as body temperature (for mammals) and performance measurements. The animals are observed and examined for at least 14 days after administration.

Unless otherwise prescribed in a specific monograph or, in the absence of a specific monograph, unless otherwise justified and authorised, the vaccine complies with the test if no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the vaccine.

1-3 SAFETY OF THE REPEATED ADMINISTRATION OF 1 DOSE

Repeated administration of 1 dose may be required to reveal any adverse effects induced by such administration. These tests are particularly important where the product, notably an immunoserum, may be administered on several occasions over a relatively short period of time. These tests are carried out on the most sensitive categories of the target species, using each recommended route of administration. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If 1 route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. The number of administrations must be not less than the maximum number recommended; for vaccines, this shall take account of the number of administrations for primary vaccination and the 1st re-vaccination; for immunosera, it shall take account of the number of administrations required for treatment. The interval between administrations shall be suitable (e.g. period of risk or required for treatment) and appropriate to the recommendations of use. Although, for convenience, as far as vaccines are concerned, a shorter interval may be used in the study than that recommended in the field, an interval of at least 14 days must be allowed between administrations for the development of any hypersensitivity reaction. For immunosera, however, administration shall follow the recommended schedule. For vaccines intended for use in mammals, in general 8 animals

per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in fish, in general 50 fish per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in birds older than 3 weeks, in general 8 birds per group are used unless otherwise justified or specified in a specific monograph. For vaccines intended for use in birds younger than 3 weeks, in general 10 birds per group are used unless otherwise justified or specified in a specific monograph. The animals are observed and examined at least daily for at least 14 days after the last administration for signs of systemic and local reactions. Other objective criteria are recorded, such as body temperature and performance measurements.

Unless otherwise prescribed in a specific monograph or, in the absence of a specific monograph, unless otherwise justified and authorised, the product complies with the test if no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the product.

1-4 EXAMINATION OF REPRODUCTIVE PERFORMANCE

When the vaccine is recommended for use or may be used in pregnant animals or laying birds, carry out a test for safety in this category of animals. If the reproductive safety studies are not performed, an exclusion statement appears on the label, unless a scientific justification for absence of risk is provided. 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 appropriate, reproductive performance of males and females and harmful effects on the progeny, including teratogenic or abortifacient effects, are investigated by each of the recommended routes of administration. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If 1 route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study.

For vaccines intended for use in mammals, in general 8 animals per group are used unless otherwise justified or specified in a specific monograph. Vaccines recommended for use or that may be used in pregnant animals, are tested in each of the specific periods of gestation recommended for use on the label. An exclusion statement will be required for those gestation periods not tested.

The observation period is extended to parturition, to examine any harmful effects during gestation or on progeny, unless otherwise justified or specified in a specific monograph.

The following protocol is given as an example of an appropriate test for vaccines.

Safety in pregnant animals Use not fewer than 8 animals per group, at the recommended stage of gestation or at a range of stages of gestation according to the recommended schedule. Not fewer than 8 animals are used for each stage of pregnancy (i.e. 24 animals for 3 trimesters of pregnancy in cattle). Administer to each animal a recommended dose of the vaccine. If the recommended schedule requires a 2nd dose, administer another dose after an interval of at least 14 days. Unless otherwise prescribed in a specific monograph, observe the animals at least daily until 1 day after parturition. Unless otherwise prescribed in a specific monograph, or, in the absence of a specific monograph, unless otherwise justified and authorised, the vaccine complies with the test if no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the vaccine, and if no adverse effects on the pregnancy or the offspring are noted.

1-5 RESIDUES

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.

1-6 ADVERSE EFFECTS ON IMMUNOLOGICAL FUNCTIONS

Where the product might adversely affect the immune response of the animal to which the product is administered or of its progeny, suitable tests on the immunological functions are carried out.

1-7 ADVERSE EFFECTS FROM INTERACTIONS

Studies are undertaken to show a lack of adverse effect on the safety of the product when simultaneous administration is recommended or where administration of the product is recommended as part of a schedule of administration of products within a short period of time.

1-8 SPECIAL REQUIREMENTS FOR LIVE VACCINES

The following laboratory tests must also be carried out with live vaccines.

For the following tests except for the test for increase in virulence (section 1-8-3), use the vaccine strain at the least attenuated passage level that will be present between the master seed lot and a batch of vaccine.

1-8-1 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.

1-8-2 Dissemination in vaccinated animal

Faeces, urine, milk, eggs, and oral, nasal and other secretions shall be tested for the presence of the organism as appropriate. 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 and shall particularly take into account the persistence of the strain at the injection site.

1-8-3 Increase in virulence

Unless otherwise prescribed in a specific monograph or, in the absence of a specific monograph, unless otherwise justified and authorised, the following applies. This test is carried out using the master seed lot. If the quantity of the master seed lot sufficient for performing the test is not available, the lowest passage material used for the production that is available in sufficient quantity may be used. At the time of inoculation, the animals in all groups are of an age suitable for recovery of the strain. Serial passages are carried out in target animals using 5 groups of animals, unless there is justification to carry out more passages or unless the strain disappears from the test animal sooner. *In vitro* propagation may not be used to expand the passage inoculum.

The passages are carried out using animals most appropriate to the potential risk being assessed.

The initial administration is carried out using the recommended route of administration most likely to lead to reversion to virulence, using an initial inoculum containing the maximum release titre. After this, not fewer than 4 further serial passages through animals of the target species are undertaken. The passages are undertaken by the route of administration most likely to lead to reversion to virulence. If the properties of the strain allow sequential passage via natural spreading, this method may be used, otherwise passage as described in each specific monograph is carried out and the micro-organisms that have been recovered at the final passage are tested for increase in virulence. For the first 4 groups, a minimum of 2 animals is used for mammalian vaccines, and a minimum of 5 birds is used for avian vaccines. The last group consist of a minimum of 8 mammals or 10 birds. At each passage, the presence of living vaccine-derived micro-organisms in the material used for passage is demonstrated. Care must be taken to avoid contamination by micro-organisms from previous passages. When the micro-organism is not recovered from any intermediate *in vivo* passage, repeat the passage in 10 animals using *in vivo* passaged material from the last passage in which the micro-organism was recovered. The micro-organism recovered is used as the inoculum for the next passage. If the target micro-organism is not recovered, the experiment is considered to be completed with the conclusion that the target micro-organism does not show an increase in virulence.

General clinical observations are made during the study. Animals in the last group are observed for 21 days unless otherwise justified. These observations include all relevant parameters typical for the disease that could indicate increase in virulence. Compare the clinical signs and other relevant parameters with those observed in the animals used in the test for safety of the administration of 1 dose (section 1-1). If the last group of animals shows no evidence of an increase in virulence, further testing is not required. Otherwise, material used for the 1st passage and the micro-organisms recovered at the final passage level are used in a separate experiment using at least 8 animals per group for mammal vaccines and at least 10 birds per group for avian vaccines, to compare directly the clinical signs and other relevant parameters. This study is carried out using the route of administration that was used for previous passages. An alternative route of administration may be used if justified.

Unless otherwise justified and authorised, the product complies with the test if no animal dies or shows signs attributable to the vaccine strain and no indication of increased virulence is observed in the animals of the last group.

1-8-4 Biological properties of the vaccine strain

Other tests may be necessary to determine as precisely as possible the intrinsic biological properties of the vaccine strain (for example, neurotropism). For vector vaccines, evaluation is made of the risk of changing the tropism or virulence of the strain and where necessary specific tests are carried out. Such tests are systematically carried out where the product of a foreign gene is incorporated into the strain as a structural protein.

1-8-5 Recombination or genomic reassortment of strain.

The probability of recombination or genomic reassortment with field or other strains shall be considered.

2 FIELD STUDIES

Results from laboratory studies shall normally be supplemented with supportive data from field studies. Provided that laboratory tests have adequately assessed the safety and efficacy of a product under experimental conditions using vaccines of maximum and minimum titre or potency respectively, a single batch of product may be used to assess both safety and efficacy under field conditions. In these cases, a typical routine batch of intermediate titre or potency may be used.

For food-producing mammals, the studies include measurement of the body temperatures of a sufficient number of animals, before and after administration of the product; for other mammals, such measurements are carried out if the laboratory studies indicate that there might be a problem. The size and persistence of any local reaction and the proportion of animals showing local or systemic reactions are recorded. Performance measurements are made, where appropriate.

Performance measures for broilers include weekly mortality, feed conversion ratios, age at slaughter and weight, down grading and rejects at the processing plant. For vaccines for use in laying birds or in birds that may be maintained to lay, the effect of the vaccine on laying performance and hatchability is investigated, as appropriate.

3 ECOTOXICITY

An assessment is made of the potential harmful effects of the product for the environment and any necessary precautionary measures to reduce such risks are identified. The likely degree of exposure of the environment to the product is assessed, taking into account: the target species and mode of administration; excretion of the product; and disposal of unused product. If these factors indicate that there will be significant exposure of the environment to the product, the potential ecotoxicity is evaluated, taking into account the properties of the product.