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

Veterinary Vaccines

General Notices

(Vaccines for Veterinary Use, Ph. Eur. monograph 0062)

Veterinary Vaccines comply with the requirements of the European Pharmacopoeia monograph for Vaccines for Veterinary Use. These requirements are reproduced below.

The provisions of this monograph apply to the following vaccines.

Inactivated Bacterial Vaccines

Bovine Leptospirosis Vaccine (Inactivated)*

Canine Leptospirosis Vaccine (Inactivated)*

Clostridium Botulinum Vaccine*

Clostridium Chauvoei Vaccine*

Clostridium Novyi Type B Vaccine*

Clostridium Perfringens Vaccines*

Clostridium Septicum Vaccine*

Clostridium Tetani Vaccines*

Feline Chlamydiosis Vaccine (Inactivated)*

Fowl Cholera Vaccine (Inactivated)*

Furunculosis Vaccine for Salmonids, Inactivated*

Mannheimia Vaccine (Inactivated) for Cattle*

Mannheimia Vaccine (Inactivated) for Sheep*

Mycoplasma Gallisepticum Vaccine (Inactivated)*

Pasteurella Vaccine (Inactivated) for Sheep*

Porcine Actinobacillosis Vaccine, Inactivated*

Porcine Enzootic Pneumonia Vaccine (Inactivated)*

Porcine E. Coli Vaccine, Inactivated*

Porcine Progressive Atrophic Rhinitis Vaccine, Inactivated*

Ruminant E. Coli Vaccine, Inactivated*

Salmonella Enteritidis Vaccine (Inactivated) for Chickens*

Salmonella Typhimurium Vaccine (Inactivated) for Chickens*

Swine Erysipelas Vaccine, Inactivated*

Vibriosis Vaccine for Salmonids, Inactivated, Cold-water*

Vibriosis Vaccine for Salmonids, Inactivated*

Vibriosis Vaccine for Sea Bass, Inactivated*

Living Bacterial Vaccines

Anthrax Vaccine, Living*

Brucella Melitensis (Strain Rev. 1) Vaccine, Living*

Coccidiosis Vaccine (Live) for Chickens*

Salmonella Dublin Vaccine, Living

Inactivated Viral Vaccines

Aujesky's Disease Vaccine, Inactivated*

Avian Infectious Bronchitis Vaccine, Inactivated*

Avian Paramyxovirus 3 Vaccine, Inactivated*

Bovine Viral Diarrhoea Vaccine (Inactivated)*

Calf Coronavirus Diarrhoea Vaccine (Inactivated)*

Calf Rotavirus Diarrhoea Vaccine (Inactivated)*

Canine Adenovirus Vaccine, Inactivated*

Canine Parvovirus Vaccine, Inactivated *

Egg-drop Syndrome 76 (Adenovirus) Vaccine*

Equine Herpesvirus Vaccine, Inactivated*

Equine Influenza Vaccine*

Feline Calicivirus Vaccine, Inactivated*

Feline Infectious Enteritis Vaccine, Inactivated*

Feline Leukaemia Vaccine, Inactivated*

Feline Viral Rhinotracheitis Vaccine, Inactivated*

Foot and Mouth Disease (Ruminants) Vaccine*

Infectious Bursal Disease Vaccine, Inactivated*

Louping-ill Vaccine

Newcastle Disease Vaccine, Inactivated*

Ovine Enzootic Abortion Vaccine

Porcine Parvovirus Vaccine, Inactivated*

Rabbit Haemorrhagic Disease Vaccine (Inactivated)*

Rabies Veterinary Vaccine, Inactivated*

Swine Influenza Vaccine, Inactivated*

Living Viral Vaccines

Aujesky's Disease Vaccine, Living*

Avian Infectious Bronchitis Vaccine, Living*

Avian Viral Tenosynovitis Vaccine (Live)*

Bovine Parainfluenza Virus Vaccine, Living*

Bovine Respiratory Syncytial Virus Vaccine, Living*

Canine Adenovirus Vaccine, Living *

Canine Distemper Vaccine, Living*

Canine Parainfluenza Virus Vaccine (Live)*

Canine Parvovirus Vaccine, Living*

Contagious Pustular Dermatitis Vaccine, Living

Duck Plague Vaccine (Live)*

Duck Viral Hepatitis Type I Vaccine (Live)*

Feline Calicivirus Vaccine, Living*

Feline Infectious Enteritis Vaccine, Living*

Feline Viral Rhinotracheitis Vaccine, Living*

Ferret and Mink Distemper Vaccine, Living*

Fowl Pox Vaccine, Living*

Infectious Avian Encephalomyelitis Vaccine, Living*

Infectious Bovine Rhinotracheitis Vaccine, Living*

Infectious Bursal Disease Vaccine, Living*

Infectious Chicken Anaemia Vaccine (Live)*

Laryngotracheitis Vaccine, Living*

Marek's Disease Vaccine, Living*

Myxomatosis Vaccine (Live) for Rabbits*

Newcastle Disease and Avian Infectious Bronchitis Vaccine, Living

Newcastle Disease Vaccine, Living*

Rabies Vaccine for Foxes, Living*

Swine-Fever Vaccine (Live, Prepared in Cell Cultures), Classical*

Helminth Vaccine

Lungworm (Dictyocaulus Viviparus) Oral Vaccine, Living

*Monograph of the European Pharmacopoeia

Ph Eur

In the case of combined vaccines, for each component that is the subject of a monograph in the Pharmacopoeia, the provisions of that monograph apply to that component, modified where necessary as indicated (see general chapters 5.2.6. Evaluation of safety of veterinary vaccines and immunosera and 5.2.7. Evaluation of efficacy of veterinary vaccines and immunosera).

If an immunological product for veterinary use is intended for minor use, certain tests may be excluded or their protocols adapted, subject to approval by the competent authority[1].

1 DEFINITION

Vaccines for veterinary use are preparations containing antigenic substances and are administered for the purpose of inducing a specific and active immunity against disease provoked by bacteria, toxins, viruses, fungi or parasites. The vaccines, live or inactivated, confer active immunity that may be transferred passively via maternal antibodies against the immunogens they contain and sometimes also against antigenically related organisms. Vaccines may contain live or

inactivated micro-organisms (bacteria, viruses or fungi), parasites, antigenic fractions or substances produced by these organisms (e.g. toxins), rendered harmless whilst retaining all or part of their antigenic properties; vaccines may also contain combinations of these constituents. The antigens may be produced by biotechnology. Suitable adjuvants may be included to enhance the immunising properties of the vaccines.

The terminology used in monographs on vaccines for veterinary use is defined in general chapter 5.2.1.

1-1 BACTERIAL VACCINES AND BACTERIAL TOXOIDS

Bacterial vaccines and bacterial toxoids are prepared from cultures grown on suitable solid or liquid media, or by other suitable means; the requirements of this section do not apply to bacterial vaccines prepared in cell cultures or in live animals. The strain of bacterium used may have been modified by genetic engineering. The identity, antigenic potency and purity of each bacterial culture used is carefully controlled.

Bacterial vaccines contain inactivated or live bacteria or their antigenic components; they are either liquid preparations of varying degrees of opacity or they may be freeze-dried.

Bacterial toxoids are prepared from toxins by diminishing their toxicity to a very low level or by completely eliminating it by physical or chemical means whilst retaining adequate immunising potency. The toxins are obtained from selected strains of specified micro-organisms grown in suitable media or are obtained by other suitable means, e.g. chemical synthesis.

Bacterial toxoids are clear or slightly opalescent liquids. Adsorbed toxoids are suspensions or emulsions. Certain toxoids may be freeze-dried.

Unless otherwise indicated, statements and requirements given below for bacterial vaccines also apply to bacterial toxoids and products containing a combination of bacterial cells and toxoids.

1-2 VIRAL VACCINES

Viral vaccines are prepared by growth in suitable cell cultures (5.2.4), in tissues, in micro-organisms, in embryonated eggs or, where no other possibility is available, in live animals, or by other suitable means. The strain of virus used may have been obtained or modified by genetic engineering, or synthesised. They are either liquid, frozen or freeze-dried preparations of 1 or more viruses or viral subunits or peptides.

Live viral vaccines are prepared from viruses of attenuated virulence or of natural low virulence for the target species.

Inactivated viral vaccines are treated by a validated procedure for inactivation.

Both live and inactivated virus harvests may be purified and concentrated.

1-3 VECTOR VACCINES

Vector vaccines are liquid or freeze-dried preparations of 1 or more types of live micro-organisms (bacteria, viruses or fungi) that are non-pathogenic or have low pathogenicity for the target species and in which have been inserted 1 or more genes encoding antigens that stimulate an immune response protective against other micro-organisms.

2 PRODUCTION

General provisions

Production is designed to provide a finished product that complies with the approved requirements. Compliance with these requirements is demonstrated by safety and efficacy studies carried out on batches during development and by the control strategy. The tests to be applied are outlined below and in individual monographs. In accordance with the General Notices, performance of all the tests in a monograph is not necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. Therefore, routinely used *in vivo* tests can ultimately be replaced in accordance with the principles of the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*, if the product profile is well defined by a set of parameters, including antigen content and antigen quality, established to verify that the manufacturing process consistently produces final batches equivalent to a final batch that fulfils the criteria of the European Pharmacopoeia.

When appropriate, production is designed to provide a finished product that does not interfere with national disease eradication programmes.

2-1 STARTING MATERIAL

2-1-1 Substrates for production

Cell cultures used for the production of vaccines for veterinary use comply with the requirements of general chapter *5.2.4*.

Where vaccine organisms are grown in embryonated hens' eggs, such eggs are derived either from SPF flocks (*5.2.2*) or from healthy non-SPF flocks (*5.2.13*).

Where vaccine organisms are grown in embryonated eggs other than hens' eggs, the requirements of chapter *5.2.5* section 4-1-1-2-3. Animals should be considered for the birds from which the eggs are sourced.

Where it is unavoidable to use animals or animal tissues in the production of vaccines, the requirements of chapter *5.2.5*. section 4-1-1-2-3. Animals apply.

General requirements for managing the presence of extraneous agents in substrates for production are given in general chapter *5.2.5. Management of extraneous agents in immunological veterinary medicinal products*.

2-1-2 Media used for seed culture preparation and for production

Media used for seed culture preparation and for production are prepared following a standard formulation. The medium is considered a starting material, and its composition is specified in the description of the manufacturing process. The qualitative and quantitative composition of all media used must be recorded. Ingredients that are derived from animals are specified in terms of the source species and region or country of origin, and must comply with the criteria described in general chapter *5.2.5*. Preparation processes for media used, including sterilisation procedures, are documented.

The addition of antibiotics during the manufacturing process is normally restricted to cell culture fluids and other media, egg inocula and material harvested from tissues and embryonated eggs. During vaccine development any addition of antibiotics is evaluated, taking into account the type, number and amount of antibiotics.

2-1-3 Seed lots

2-1-3-1 Bacterial seed lots

2-1-3-1-1 General requirements. The genus and species (and varieties where appropriate) of the bacteria used in the vaccine are stated. Bacteria used in manufacture are handled in a seed-lot system wherever possible. Each master seed lot is tested as described below. A record of the storage conditions is maintained for each master seed lot. Each master seed lot is assigned a specific code for identification purposes.

2-1-3-1-2 Propagation. The minimum and maximum numbers of subcultures of each master seed lot prior to the production stage are specified. The methods used for the preparation of seed cultures, preparation of suspensions for seeding, techniques for inoculation of seeds, titre and concentration of inocula and the media used, are documented. It shall be demonstrated that the characteristics of the seed material (e.g. dissociation or antigenicity) are not changed by these subcultures. The conditions under which each seed lot is stored are documented.

2-1-3-1-3 Identity and purity. Each master seed lot is shown to contain only the species and strain of bacterium stated. A brief description of the method of identifying each strain by its biochemical, serological, morphological or other appropriate characteristics and distinguishing it as far as possible from related strains is recorded, as is the method of determining the purity of the strain.

2-1-3-2 Virus seed lots

2-1-3-2-1 General requirements. Viruses used in manufacture are handled in a seed-lot system. Each master seed lot is tested as described below. A record of the storage conditions is maintained for each seed lot. Each master seed lot is assigned a specific code for identification purposes. Vaccine production is not undertaken using a virus more than 5 passages from the master seed lot, unless otherwise justified. In the tests on the master seed lot described below, the material tested is not more than 5 passages from the master seed lot at the start of the tests, unless otherwise indicated.

Where the master seed lot is contained within a permanently infected master cell seed, the following tests are carried out on an appropriate volume of virus from disrupted master cell seed. Where relevant tests have been carried out on disrupted cells to validate the suitability of the master cell seed, these tests need not be repeated.

2-1-3-2-2 Propagation. The master seed lot and all subsequent passages are propagated on cells, on embryonated eggs or in animals that have been shown to be suitable for vaccine production and, where applicable, using substances of animal origin that meet the requirements prescribed in general chapter 5.2.5.

2-1-3-2-3 Identification. A suitable method to identify the vaccine strain and to distinguish it as far as possible from related strains must be used.

2-1-3-2-4 Bacteria and fungi. The master seed lot complies with the test for sterility (2.6.1).

2-1-3-2-5 Mycoplasmas (2.6.7). The master seed lot complies with the test for mycoplasmas.

2-1-3-2-6 Absence of extraneous viruses. General requirements for managing the presence of extraneous viruses in master seed lots are given in general chapter 5.2.5.

2-1-4 Substances

Where applicable, substances used for the production of vaccines for veterinary use comply with the requirements of the relevant monographs and the general requirements for managing the presence of extraneous agents given in general chapter 5.2.5. They are prepared in a manner that avoids contamination of the vaccine.

2-2 CHOICE OF VACCINE STRAIN AND COMPOSITION

When deciding on the strain to be included in the vaccine, and the overall vaccine composition, safety, efficacy and stability are critical aspects to be taken into account.

2-2-1 Development studies on safety and efficacy

General requirements for evaluation of safety and efficacy are given in general chapters 5.2.6 and 5.2.7. These requirements may be made more explicit or supplemented by the requirements of individual monographs.

2-2-1-1. *Potency and immunogenicity*. The tests given under the headings Potency and Immunogenicity in monographs serve 2 purposes:

— the Potency section establishes, by a well-controlled test in experimental conditions, the minimum acceptable vaccinating capacity for all vaccines within the scope of the definition, which must be guaranteed throughout the shelf life;

— well-controlled experimental studies are normally a part of the overall demonstration of efficacy of a vaccine (see general chapter 5.2.7): the test referred to in the Immunogenicity section (to which the Potency section usually cross-refers) is suitable as part of this testing.

2-2-1-2 Information on performing the safety and efficacy studies. During development of a vaccine, safety and immunogenicity are demonstrated for each route and for each method of administration to be recommended. The following is a non-exhaustive list of such routes of administration:

— intramuscular;

— subcutaneous;

— intravenous;

— ocular;

— oral;

— nasal;

— foot-stab;

— wing web;

— intradermal;

— intraperitoneal;

— *in ovo*.

The following is a non-exhaustive list of such methods of administration:

- — injection;
- — drinking water;
- — spray;
- — eye-drop;
- — scarification;
- — implantation;
- — immersion.

Monographs may indicate that a given test is to be carried out for each category of animal of the target species for which the product is recommended or is to be recommended. The following is a non-exhaustive list of categories that are to be taken into account.

- — *Mammals*:
  - — pregnant animals/non-pregnant animals;
  - — animals raised primarily for breeding/animals raised primarily for food production;
  - — animals of the minimum age or size recommended for vaccination.
- — *Avian species*:
  - — birds raised primarily for egg production/birds raised primarily for production of meat;
  - — birds before point of lay/birds after onset of lay.
- — *Fish*:
  - — broodstock fish/fish raised primarily for food production.

2-2-2 Antimicrobial preservatives

Antimicrobial preservatives are used to prevent spoilage or adverse effects caused by microbial contamination occurring during use of a vaccine which is expected to be no longer than 10 h after first broaching. Antimicrobial preservatives are not included in freeze-dried products but, if justified, taking into account the maximum recommended shelf life after reconstitution, they may be included in the diluent for multidose freeze-dried products. For single-dose liquid preparations, inclusion of antimicrobial preservatives is not acceptable unless justified and authorised, but may be acceptable, for example where the same vaccine is filled in single-dose and multidose containers and is used in non-food-producing species. For multidose liquid preparations, the need for effective antimicrobial preservation is evaluated taking into account likely contamination during use and the maximum recommended shelf life after broaching of the container.

During development studies the effectiveness of the antimicrobial preservative throughout the shelf life shall be demonstrated to the satisfaction of the competent authority.

The efficacy of the antimicrobial preservative is evaluated as described in general chapter *5.1.3* and in addition, samples are tested at suitable intervals over the proposed in-use shelf life. If neither the A criteria nor the B criteria can be met, then in justified cases the following criteria are applied to vaccines for veterinary use: bacteria, no increase from 24 h to 7 days, 3 $\log_{10}$ reduction at 14 days, no increase at 28 days; fungi, no increase at 14 days and 28 days.

Addition of antibiotics as antimicrobial preservatives is generally not acceptable.

2-2-3 Stability

Evidence of stability is obtained to justify the proposed shelf life. This evidence takes the form of the results of virus titrations, bacterial counts or potency tests carried out at regular intervals until 3 months beyond the end of shelf life on not fewer than 3 representative consecutive batches of vaccine kept under recommended storage conditions together with results from studies of moisture content (for freeze-dried products), physical tests on the adjuvant, chemical tests on substances such as the adjuvant constituents and preservatives, and pH, as appropriate.

Where applicable, studies on the stability of the reconstituted vaccine are carried out, using the product reconstituted in accordance with the proposed recommendations.

The variations in the results obtained during the stability study are taken into account when defining appropriate formulation and release specifications to ensure the conformity of the product for the claimed shelf life.

2-2-4 Formulation

The minimum antigen content, virus titre or bacterial count acceptable from the point of view of efficacy (i.e. gives satisfactory results in the potency test and other efficacy studies) is established during development studies. The antigen formulation, where applicable the adjuvant formulation, and the release specifications are set based on this minimum value and based on the results of the stability studies.

A maximum antigen content, virus titre or bacterial count, acceptable from the point of view of safety, is established during development studies.

For live vaccines, this is also used as the maximum acceptable titre for each batch of vaccine at release.

2-3 PREPARATION OF THE VACCINE

The methods of preparation, which vary according to the type of vaccine, are such as to maintain the integrity and immunogenicity of the antigen, to ensure freedom from contamination with extraneous agents and to ensure production of vaccine batches of consistent quality.

For each individual product, relevant in-process and finished product controls are established to verify the production process and the batch-to-batch quality of the product. The results are within the approved limits defined for the particular product.

2-3-1 Propagation and harvest of bacterial and viral antigens

Each strain of a multivalent vaccine is cultivated and harvested separately.

The working seed materials are propagated in suitable media/substrates for production. The conditions of these propagation steps are described and monitored by recording appropriate parameters, e.g. temperature, pH, duration, turbidity and oxygen saturation. The results are within the approved limits defined for the particular product.

During production, where possible, growth rate is monitored by suitable methods and the values are recorded and are within the approved limits defined for the particular product. The antigen may then be inactivated and/or purified and/or concentrated.

2-3-2 Inactivation

Inactivated vaccines are subjected to a validated inactivation procedure. When conducting tests for inactivation, it is essential to take into account the possibility that under the conditions of manufacture, organisms may be physically protected from inactivation.

2-3-2-1 Inactivation kinetics. Testing for inactivation kinetics, as described below, is carried out once for a given production process. The inactivating agent and the inactivation procedure shall be shown, under conditions of manufacture, to inactivate the vaccine micro-organism. Adequate data on inactivation kinetics shall be obtained. Normally, the time required for inactivation shall be not more than 67 per cent of the duration of the inactivation process. The maximum titre of the vaccine micro-organism targeted by the selected inactivation method is established based on the inactivation kinetics data.

2-3-2-2 Residues of inactivating and detoxifying agents. Appropriate tests on each production run or validations are carried out to demonstrate that the inactivating or detoxifying agent has been removed, neutralised or reduced to an acceptable residual level.

If an aziridine compound is used as the inactivating agent, this may be accomplished by neutralising it with thiosulfate and demonstrating residual thiosulfate in the inactivated harvest at the completion of the inactivation procedure.

If formaldehyde is used as the inactivating agent, a test for free formaldehyde is carried out as prescribed under section 3. Batch tests.

2-3-2-3 Residual live virus/bacteria and/or detoxification testing. For each production run, a test for complete inactivation and/or detoxification is performed immediately after the inactivation and/or detoxification procedure or after subsequent process steps enhancing the sensitivity of the test (e.g. concentration step). Validation of the test for residual live virus/bacteria or the test for detoxification shall focus on the level of detection of the live virus/bacteria or toxin.

2-3-2-3-1 Bacterial vaccines. The test selected shall be appropriate for the vaccine bacteria used and shall consist of at least 2 passages in production medium or, if solid medium has been used for production, in a suitable liquid medium or in the medium prescribed in the monograph. The product complies with the test if no evidence of any live micro-organism is observed.

2-3-2-3-2 Bacterial toxoids. The test selected shall be appropriate for the toxin or toxins present and shall be the most sensitive available.

2-3-2-3-3 Viral vaccines. The test selected shall be appropriate for the vaccine virus being used and must consist of at least 2 passages in cells, embryonated eggs or, where no other suitably sensitive method is available, in animals. The quantity of cell samples, eggs or animals shall be sufficient to ensure appropriate sensitivity of the test. For tests in cell cultures, not less than 150 cm^2 of cell culture monolayer is inoculated with 1.0 mL of inactivated harvest. The product complies with the test if no evidence of the presence of any live virus or other micro-organism is observed.

2-3-3 Final bulk and final batch

The final bulk vaccine is prepared by combining 1 or more batches of antigen, which comply with the relevant requirements, with other substances such as adjuvants, stabilisers, antimicrobial preservatives and diluents. Batches of live bacterial antigen are tested for purity. Any other types of antigen and all substances are sterile unless otherwise justified and authorised.

The vaccine is blended according to a defined formulation.

Unless otherwise prescribed in the individual monograph or otherwise justified and authorised, the final bulk vaccine is filled and distributed aseptically with or without freeze-drying, into sterile, tamper-evident containers, which are then closed so as to prevent contamination.

For non-liquid vaccines for administration by a non-parenteral route only, in agreement with the competent authority, the final bulk vaccine may be filled and distributed under suitable conditions into suitable tamper-evident containers.

This constitutes the final batch.

2-4 MANUFACTURER'S TESTS

2-4-1 Antigen content

The formulation of the vaccine is based, whenever possible, on the antigen content determined on the harvest before or after inactivation and/or downstream processing, if applicable.

2-4-2 Batch potency test

For most vaccines, the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches.

If the test described under Potency is not used for routine testing, a batch potency test is established during development. The aim of the batch potency test is to ensure that each batch of vaccine would, if tested, comply with the test described under Potency and Immunogenicity. The acceptance criteria for the batch potency test are therefore established by correlation with the test described under Potency. Where a batch potency test is described in a monograph, this is given as an example of a test that is considered suitable, after establishment of correlation with the potency test; other test models can also be used.

For live vaccines, virus titre or bacterial count is generally appropriate as a batch potency test.

For inactivated vaccines, development of *in vitro* methods is recommended, provided that:

— key in-process parameters are defined and monitored;

— in-process control tests (including antigen quantification after inactivation and/or concentration, if applicable) and target formulation of the final product are performed.

Antigen content The quantity of appropriate antigen per dose, determined by a suitable method, is not significantly lower than that of a batch of vaccine that has given satisfactory results in the test described under Potency.

Adjuvant If the test for antigen content is performed and if the vaccine is adjuvanted, the identity of the adjuvant is verified by suitable chemical methods and the adjuvant is tested as described in section 3. Batch tests. The quality and

quantity of the adjuvant is not significantly different from that of a batch of vaccine that has given satisfactory results in the test described under Potency.

2-5 IN-PROCESS STABILITY

During production of vaccines, intermediate products are obtained at various stages and may be stored. The intended conditions and duration of storage are defined in light of the stability data.

3 BATCH TESTS

The individual monographs also indicate tests to be carried out on each particular vaccine.

Certain tests may be carried out on the final bulk vaccine rather than on the batch or batches prepared from it; these include tests for antimicrobial preservatives and free formaldehyde and the potency determination for inactivated vaccines.

Under particular circumstances (i.e. significant changes to the manufacturing process, as well as reports of unexpected adverse reactions observed in the field or reports that the final batches do not comply with the former data provided during licensing), other tests, including tests on animals, may be needed on an *ad hoc* basis; they are carried out in agreement with or at the request of the competent authority. For safety testing, one or more of the tests described in general chapter *5.2.6* may be carried out.

Only a batch that complies with each of the requirements given below, completed or amended by the requirements given in the relevant individual monograph, may be released for use.

Animal tests In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The criteria for judging tests in monographs must be applied in light of this. For example, if it is indicated that an animal is considered to be positive, infected etc., when typical clinical signs occur then as soon as it is clear that the result will not be affected, the animal in question shall be either euthanised or given suitable treatment to prevent unnecessary suffering. In accordance with the General Notices, alternative test methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animal use or a reduction of suffering.

Guidance on how to substitute *in vivo* methods by *in vitro* methods where a direct head-to-head comparison is not possible may be found in general chapter *5.2.14*.

Taking into account the quality systems in place and advances in scientific knowledge and understanding of the products, manufacturing processes and their controls, the choice of tests to be performed may be reconsidered when assessing compliance with Pharmacopoeia monographs, in accordance with the General Notices. On a case-by-case basis, with the agreement of the competent authority, the choice of and need for certain final product tests may be reconsidered, where in-process tests are able to demonstrate that the finished product meets the requirements of the monograph or where alternative tests validated with respect to the Pharmacopoeia method have been carried out.

All hen eggs, chickens and chicken cell cultures for use in quality control tests shall be derived from a SPF flock (*5.2.2*).

3-1 Identification

The antigen is identified using suitable methods.

3-2 Physical tests

A vaccine with an oily adjuvant is tested for viscosity by a suitable method and shown to be within the limits set for the product. The stability of the emulsion shall be demonstrated.

3-3 Chemical tests

Tests for the concentrations of appropriate substances such as aluminium and preservatives are carried out to show that these are within the limits set for the product.

3-4 pH

The pH of liquid products and diluents is measured if possible and shown to be within the limits set for the product.

3-5 Water

Where applicable, the freeze-drying process is checked by a determination of water and shown to be within the limits set for the product.

3-6 Formaldehyde

(*2.4.18*; use Method B if sodium metabisulfite has been used to neutralise excess formaldehyde). Where formaldehyde has been used in the preparation, the concentration of free formaldehyde is not greater than 0.5 g/L, unless a higher amount has been shown to be safe.

3-7 Phenol (*2.5.15*)

When the vaccine contains phenol, the concentration is not greater than 5 g/L.

3-8 Bacteria and fungi

Vaccines comply with the test for sterility (*2.6.1*). Where the volume of liquid in a container is greater than 100 mL, the membrane filtration method is used wherever possible. Where the membrane filtration method cannot be used, the direct inoculation method may be used. Where the volume of liquid in each container is at least 20 mL, the minimum volume to be used for each culture medium is 10 per cent of the contents or 5 mL, whichever is less. The appropriate number of items to be tested (*2.6.1*) is 1 per cent of the batch with a minimum of 4 and a maximum of 10.

For live bacterial and for live fungal vaccines, the absence of micro-organisms other than the vaccine strain is demonstrated by suitable methods such as microscopic examination and inoculation of suitable media.

For frozen or freeze-dried avian live viral vaccines produced in embryonated eggs, for non-parenteral use only, the requirement for sterility is usually replaced by requirements for absence of pathogenic micro-organisms and for a maximum of 1 non-pathogenic micro-organism per dose.

For other vaccines presented in a non-liquid form for non-parenteral use only, in agreement with the competent authority and provided that the product remains stable throughout its shelf life, the requirement for sterility may be replaced by requirements for absence of relevant pathogenic micro-organisms and an appropriately low number of micro-organisms per dose, based on batch data and process validation.

3-9 Extraneous agents (*5.2.5*)

The vaccine is free from extraneous agents.

3-10 Residual live virus/bacteria and/or detoxification testing

For inactivated vaccines and bacterial toxoids, the tests described in section 2-3-2-3 are performed. Where excipients would interfere with a test for inactivation and/or detoxification, such a test is carried out during preparation of the final bulk, after the different batches of antigen have been combined, but before addition of the excipients; the test for inactivation or detoxification may then be omitted on the final bulk and the final batch.

The test for residual live virus/bacteria may be omitted for batch release provided that:

1. a titration is performed on each harvest prior to inactivation and the titre is not greater than the maximum titre established based on studies of the inactivation kinetics;
2. suitable test sensitivity for residual live virus/bacteria has been demonstrated;
3. the test for residual live virus/bacteria is performed with satisfactory results on each harvest.

Where there is a risk of reversion to toxicity, the test for detoxification performed at the latest stage of the production process at which the sensitivity of the test is not compromised (e.g. after the different batches of antigen have been combined but before the addition of excipients) is important to demonstrate a lack of reversion to toxicity.

3-11 Mycoplasmas (*2.6.7*)

Live viral vaccines comply with the test for mycoplasmas (culture method).

3-12 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (see section 2-2-1-1) when administered by a recommended route and method.

4 STORAGE

Store protected from light at a temperature of 5 ± 3 °C, unless otherwise indicated. Liquid preparations are not to be allowed to freeze, unless otherwise indicated.

Expiry date Unless otherwise stated, the expiry date is calculated from the beginning of the virus titration or bacterial count (for live vaccines) or the beginning of the potency test (for other vaccines). For combined vaccines, the expiry date is that of the component which expires first. For vaccines stored by the manufacturer at a temperature lower than that stated on the label, the stability for the entire storage period is demonstrated by an appropriate study. The expiry date is then calculated from the date that the vaccine is stored in the conditions stated on the label.

The expiry date applies to vaccines stored in the prescribed conditions.

5 LABELLING

The label states:

— that the preparation is for veterinary use;

— the volume of the preparation and the number of doses in the container;

— the route of administration;

— the type or types of bacteria (and where applicable the antigenic components) or viruses used and for live vaccines the minimum and the maximum number of live bacteria or the minimum and the maximum virus titre;

— where applicable, for inactivated vaccines, the minimum potency in International Units;

— where applicable, the name of any antimicrobial preservative or other substance added to the vaccine;

— the name of any substance that may cause an adverse reaction;

— for freeze-dried vaccines:

— the name or composition and the volume of the reconstituting liquid to be added;

— the period within which the vaccine is to be used after reconstitution;

— for vaccines with an oily adjuvant, that if the vaccine is accidentally injected into man, urgent medical attention is necessary;

— the animal species for which the vaccine is intended;

— the indications for the vaccine;

— the instructions for use;

— any contra-indications to the use of the product including any required warning on the dangers of administration of an overdose;

— the doses recommended for different species.

[1] NOTE: Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243/2006, including any subsequent revision of this document).