Veterinary Vaccines: General Requirements

Definition

Vaccines are a heterogeneous class of medicinal products containing immunogenic substances capable of inducing specific, active and protective immunity against infectious diseases. They may be prepared from bacteria, viruses, parasites or fungi and other suitable organisms or their toxins. Vaccines may contain live attenuated or avirulent or inactivated or killed micro-organisms as antigens. Some vaccines consist of antigenic fractions or substances produced by the same pathogenic organisms but rendered harmless whilst retaining their immunogenicity. Vaccines may be prepared from one species or from two or more species of microorganisms. The antigen may be produced by recombinant DNA technology.

Vaccines may be prepared by the method described in the individual monograph or by any other appropriate method provided the identity of the antigen is maintained and the preparations are free from microbial contamination and extraneous agents. Suitable adjuvants may be added during preparation of vaccines. The addition of antibiotics during the manufacturing process is normally restricted to cell culture fluids and other media, egg inocula and material harvested from skin or other tissues. A suitable bactericide /preservative may be added to vaccines, if necessary. The final products are distributed aseptically into sterile containers that are then sealed to exclude extraneous microorganisms. Unless otherwise indicated in the monograph, the final vaccine may be filled into single dose or multiple dose containers. When filled in multidose containers it is permitted to use a bactericide/ preservative.

Bacterial Vaccine

Bacterial vaccines are either suspension of live or killed bacteria or sterile antigenic extracts or derivatives of bacteria pathogenic to animals grown on suitable solid or liquid media. A bacterial vaccine means a sterile suspension of a killed culture of the microorganism from which the vaccine derives its name or a sterile extract or derivative of a microorganism, or a pure suspension of living microorganisms which have been previously made avirulent.

They may be simple vaccines prepared from one species or may be combined or polyvalent vaccines prepared by blending two or more monovalent antigens from different species or strains. Bacterial vaccines may be prepared from cultures grown on suitable solid or liquid media.

Proper Name. The proper name of any bacterial vaccine shall be the name of the microorganism from which it is made followed by the word "Vaccine" or some other name may be approved by the licensing authority. For example Anthrax Spore Vaccine, Live, Blackquarter Vaccine, Brucella abortus (Strain 19**)** Vaccine, Live.

Bacteria
Cultures used in the preparation of the vaccine should be a standard or reference strain/serotype/species being manipulated into a vaccine. They should be thoroughly tested for identity by the generally accepted tests applicable to particular microorganisms.

Preparation
Bacterial vaccines, simple or polyvalent, are prepared-from' selected cultures after careful examination for their identity, specificity, purity and antigenicity. They may be prepared in following manner.
a) Formal cultures or bacterins
b) Vaccine of bacterial products or bacterial derivatives
c) Live bacterial vaccines

Live Bacterial Vaccines
Live bacterial vaccines are prepared from avirulent or attenuated strains of the specific bacteria that are capable of stimulating immune response against pathogenic strains of the same or of antigenically related species of bacteria.

Inactivated Bacterial Vaccines
Inactivated bacterial vaccines are either prepared from bacteria or their immunogenic components that have been inactivated in a suitable way that they retain adequate immunogenicity.

Combined Vaccine
Consist of two or more monovalent vaccines of different diseases, or antigens combined by the manufacturer at the final formulation stage. Such vaccines are intended to protect against either more than one disease, or against one disease caused by different strains or serotypes of the same organism. Monovalent vaccines when combined will be known as Polyvalent vaccine. For a combined vaccine, as stated on the product label, booster doses are permissible in demonstrating a potency test in target animals.

Multicomponent vaccine
A multicomponent preparation is formulated using equal to or more than two antigens in a vaccine formulation. The different antigenic components are intended to protect against different strains or types of the same organism and/or different organisms.

Bacterial Toxoids
Bacterial toxoids are prepared from toxins by diminishing their toxicity to low level or by completely eliminating it by physical or chemical means whilst retaining adequate immunizing potency. The toxins are obtained from selected strains of specific microorganisms, grown in a suitable media devoid of agents capable of inducing undesirable immunological reactions in animals. Bacterial toxoids may be liquid or may be prepared by adsorbing on suitable agents such as aluminium phosphate, aluminium hydroxide or any other suitable adsorbents. Bacterial toxoids are clear or slightly opalescent liquids, colourless or slightly yellow. Adsorbed toxoids may be White or greyish-white suspensions or pale yellow liquids with sediment at the bottom of container. Freeze-dried preparations are greyish-white or yellowish-white powders or pellets. Unless otherwise indicated in individual monograph, statements and requirements given below for bacterial vaccines also apply to bacterial toxoids and products containing a combination of bacterial cells and toxoids.

Viral Vaccines
Viral vaccines, live or inactivated are made from any virus pathogenic to domestic animals and poultry and made from other modified viruses which have any antigenic value. A virus vaccine means sterile suspension or a freeze dried powder or frozen containing the modified living or inactivated virus particles, which in its original unaltered stage, causes disease from which the vaccine derives its name and which has been prepared by a suitable method of propagation.

Proper Name. The proper name of a viral vaccine shall be the name of the disease which is caused by a particular virus from which the vaccine is produced followed by the word "Vaccine" or some other name as approved by the licensing authority. For example Avian Infectious Bronchitis Vaccine, Inactivated, Avian Infectious Bronchitis Vaccine, Live are included in Pharmacopoeia.

Viruses
The seed virus used in the preparation of vaccine shall, before being used for preparing a batch, be thoroughly tested for identity, purity, sterility, safety and potency by generally accepted tests applicable to a particular virus. It shall not be more than five passages away from the stock seed virus, unless otherwise prescribed for a particular virus. The master seed virus shall be maintained by seed-lot system at specified passage level and tested for bacterial, mycoplasma and extraneous viral contamination.

Viral vaccines are prepared by growth in suitable cell cultures (2.7.13), 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 synthetised. 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.

Vector Vaccines

Vector vaccines are liquid or freeze-dried or frozen 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.

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 Committee for Control and Supervision of Experiments on Animals (CCSEA), 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 Indian Pharmacopoeia.

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

Starting Material

Substrates for production

Cell cultures used for the production of vaccines for veterinary use comply with the requirements of general chapter (2.7.13)

Where vaccine organisms are grown in embryonated hens' eggs, such eggs are derived either from SPF flocks (2.7.18) or from healthy non-SPF flocks (2.7.7)

Where vaccine organisms are grown in embryonated eggs other than hens' eggs, the requirements of chapter *Management of extraneous agents in immunological veterinary medicinal products* (2.7.19). 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 *Management of extraneous agents in immunological veterinary medicinal products*. (2.7.19) .Animals apply.

General requirements for managing the presence of extraneous agents in substrates for production are given in general chapter *Management of extraneous agents in immunological veterinary medicinal products (2.7.19)*.

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 *Management of extraneous agents in immunological veterinary medicinal products (2.7.19)*. Preparation processes for media used, including sterilization 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.

Seed Lots

Bacterial Seed Lots

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 passage history and storage conditions is maintained for each master seed lot. Each master seed lot is assigned a specific code or number for identification purposes.

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. The conditions under which each seed lot has to be stored are documented.

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 biochemical or molecular, serological and morphological characteristics and distinguishing it as far as possible from related strains is recorded, as is also the method of determining the purity of the strain. If the master seed lot is shown to contain living organisms of any kind other than the species and strain stated, then it is unsuitable for vaccine production. Once the master seed lot and working seed lot are identified by the above means, it is not necessary to carry out the testing on every lot of the batch produced provided traceability is established and documented by the firm. In such cases, this testing also serves the identity purposes where applicable for a batch release. However, purity needs to be shown for every lot of the batch during production stages.

Virus seed lots

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.

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 *Management of extraneous agents in immunological veterinary medicinal products* (2.7.19).

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

Sterility/ Bacterial and Fungal Contamination (2.2.11).The master seed lot complies with the test for sterility

Mycoplasmas (*2.7.8*/2.7.9). The master seed lot complies with the test for mycoplasmas.

Absence of extraneous viruses. General requirements for managing the presence of extraneous viruses in master seed lots are given in general chapter *Management of extraneous agents in immunological veterinary medicinal products (2.7.19)*.

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 (2.7.19). They are prepared in a manner that avoids contamination of the vaccine.

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.

Development studies on safety and efficacy
General requirements for evaluation of and efficacy and safety are given in general chapters (2.7.12)-Evaluation of Efficacy of vaccines and Evaluation of Safety of Veterinary Vaccines and Immunosera (2.7.17) respectively. These requirements may be made more explicit or supplemented by the requirements of individual monographs.

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 period of validity;

— well-controlled experimental studies are normally a part of the overall demonstration of efficacy of a vaccine (see general chapter 2.7.12): the test referred to in the Immunogenicity section (to which the Potency section usually cross-refers) is suitable as part of this testing.

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.

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. The addition of antibiotics during the manufacturing process is normally restricted to cell culture, fluids and other media, egg inocula and material harvested from skin or other tissues. A suitable bactericide may be added to sterile and inactivated vaccines. Antimicrobial preservatives are not included in freeze-dried products but, if justified, taking into account the maximum recommended period of use 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 period of use after broaching of the container. The final products are distributed aseptically into sterile containers that are then sealed to exclude extraneous microorganisms.

During development studies the effectiveness of the antimicrobial preservative throughout the period of validity shall be demonstrated up to the satisfaction of the National Regulatory Authority.
The efficacy of the antimicrobial preservative is evaluated as described in general chapter (2.2.2).
Addition of antibiotics as antimicrobial preservatives is generally not acceptable.

Stability

Stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf life. Evidence of stability is obtained to justify the proposed period of validity. This evidence takes the form of the results of virus titrations, bacterial counts or potency tests carried out at regular intervals as per suitable regulatory guidelines available in the country 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.

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.

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.

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.

Inactivation

Inactivated vaccines are subjected to validated inactivation procedure. The testing of inactivation kinetics described below is carried out once for given inactivation process. 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.

Inactivation kinetics.
The inactivating agent and inactivation procedure shall be shown, under conditions of manufacture, to inactivate the vaccine micro-organisms. 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 inactivation process. Once inactivation kinetics is

established for each applicable vaccine, it can be omitted as a test during the bioprocess unless otherwise stated in the individual monograph.

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 Batch tests.

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.

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.

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

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.

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.

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 distributed aseptically with or without freeze-drying, into sterile, tamper-evident containers, which are then closed so as to prevent contamination. This constitutes the final batch.

Manufacturer's tests

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.

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 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.

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.

BATCH TESTS

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 -Evaluation of Safety of Veterinary Vaccines and Immunosera (2.7.17) 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 Committee for Control and Supervision of Experiments on Animals (CCSEA), 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. 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 IP general chapter. Substitute *in-vivo* methods by *in-vitro* methods (2.7.20)

All hen eggs, chickens and chicken cell cultures for use in quality control tests shall be derived from a SPF flock (2.7.18).

Vaccine complies with the tests prescribed in the individual monographs including, where applicable, the following.

Identification

The antigen is identified using suitable methods. Perform antigen detection by suitable method such as in-vitro test or molecular method

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.

Aluminium *(if present)* (2.3.9). Not more than 1.25 mg of aluminium (Al) per single dose, unless otherwise stated.

Calcium *(ifpresent)* (2.3.11). Not more than 1.3 mg of Calcium (Ca) per single dose, unless otherwise stated.

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

Water (2.3.43). For freeze-dried vaccines, not more than 3.0 per cent, unless otherwise stated.

Free formaldehyde *(if present)* (2.3.20). Not more than 0.05 per cent of free formaldehyde is present in the final product, unless otherwise stated.

Phenol *(if present)* (2.3.36). Not more than 0.5 per cent is present in the final product, unless otherwise stated.

Thiomersal (2.2.12) (2.3.48). Where thiomersal has been used in the preparation of the vaccine, not more than 0.02 percent w/v, unless otherwise stated

Test for purity for live bacterial vaccines. Petri-dishes, containing suitable media are streaked with the final product and incubated at 37° for 72 hours to 120 hours. The vaccine passes the test if no growth of micro-organisms other than those from which the vaccine was prepared is observed

Viable count for living bacterial vaccines. As described in the individual monograph, the vaccine when plated on suitable medium should show presence of minimum number of viable bacteria of the strain used at the time of bottling and at any time before -Expiry.

Pyrogen. Unless otherwise stated in the individual monograph, when the volume to be injected in a single dose is 10 ml or more, injections comply with the tests for pyrogens (2.2.8), otherwise the test for bacterial endotoxins (2.2.3) is prescribed.

Dyes. Approved dye may be used in sterile diluents for monitoring identification of vaccinated animals. Use of dye should be supported by stability of the vaccine(s) intended for reconstitution with the diluents.

Residual Live Virus/Bacteria Testing. The test for complete inactivation is performed after completion of inactivation. The test shall be appropriate to the vaccine bacteria/virus being used and must consist of atleast two passages in appropriate solid / liquid media, cells, embryonated eggs or where no other suitable method is available, in animals. The quantity of cell samples, eggs or animals shall be sufficient to ensure appropriate: sensitivity of test. For test in cell cultures, not less 150cm^2 of cell culture monolayer is inoculated with 1.0ml of inactivated bulk antigen unless otherwise stated in the individual monograph. The product complies with the test, if no evidence/presence of live virus or other microorganisms is observed.

Mycoplasmas (2.7.8/2.7.9)

Live viral vaccines comply with the test for mycoplasmas

Extraneous agents (2.7.19)

The vaccine is free from extraneous agents. Monograph prescribes set of measures that taken together give an acceptable degree of assurance that the final product does not contain infectious extraneous agents. These measures includes:

1) Production within seed lot system and cell Seed system, wherever possible.
2) Extensive testing of seed lots and cell seed for extraneous agents.
3) Requirements for SPF flocks used for providing substrate for vaccine production
4) Testing of substances of animal origin, which must wherever possible, undergo inactivation procedure.
5) For live vaccines, testing of final product for infectious extraneous agents, such tests are less extensive than those carried out at earlier stages because of guarantees given by in-process testing.

Abnormal Toxicity. Where stated in the individual monograph, For non avian vaccines following tests may be used Inject 0.5ml subcutaneously into each of five mice and 2 ml intraperitoneally into each of two guinea pigs. If the vaccine being examined contains an adjuvant, inject one dose of the vaccine subcutaneously / intramuscularly into each guinea pig. Observe the animals for 7 days. None of the animals shows significant local or systemic reaction. If one animal dies or shows signs of ill health during the observation period, repeat the test. None of the animals in repeat test shall die or show signs of ill health. For avian vaccines safety test in target animals is used .

Sterility/ Bacterial and Fungal Contamination (2.2.11). Unless otherwise stated in the individual monograph, inoculate and incubate the test samples in the media for not less than 14 days at 30° to 35° in the test for detecting bacteria and at 20 to 25° in the test for detecting fungi. However, for live bacterial vaccines growth of the organisms from which the vaccine was prepared is permitted. The number of containers to be drawn for the test should be 1 percent of the containers in a batch, with minimum of 3 and a maximum of 10 assuming that the preparation has been manufactured under appropriately validated conditions designed to exclude contamination.

For avian live viral vaccines, 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 one (1) non-pathogenic micro-organism per dose.

Safety. Carry out the test for each route and method of administration recommended for vaccination and in animals of each category for which the vaccine is intended or in laboratory animals, (using in each case [animals] not older or younger than the minimum age recommended for vaccination) as specified in individual monograph. To be done with equal to or lesser passage level (least attenuated stage) compared to commercial batch. To be done with highest expected dose level or [number] times higher microbial count of single dose.
For those circumstances when in vivo batch tests are conducted in target animals for reasons other than the target animal safety test (e.g. potency tests) and these tests include the collection of safety information (e.g. on mortality), it is recommended that manufacturers use these tests to gain additional data of the safety of the vaccine in the target species.

Note: The batch safety test using target animal may be omitted if 1] safety test has been performed with satisfactory results in master seed lot and 2] consistency of manufacturing process has been well established up to the satisfaction of NRA and 3] at least 10 consecutive production batches have been produced and comply with the safety test. Significant changes to the manufacturing process may require resumption of routine safety testing to re-establish consistency.

Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity when administered by a recommended route and method.

Determine the potency of the vaccine using the method described in the individual monograph. The vaccine complies with the level of immune response specified in the monograph. A combined vaccine complies with the level specified in the respective monographs for each individual component. If the immunogenicity (Potency test) has been performed with satisfactory results on representative batch of live vaccines from the same seed lot, it may omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot or a suitable validated alternate in vitro test may be used with approval of National Regulatory Authority. For any inactivated vaccines, unless otherwise stated in the monograph, potency challenge tests are not necessary if a validated alternate in vitro method can be demonstrated for the intended use in the animals.

Titre. Vaccine (indigenous or imported) for which IP Monograph is available, shall comply optimum titre as mentioned in the monograph to avoid any controversy and legal implications, however vaccine for which IP monographs is not available, it should be justified with scientific data or country of origin approval or both.

STORAGE

Liquid vaccines must be stored at a temperature between 2° to 8° and should not be allowed to freeze unless otherwise specified in the individual monograph. Freeze dried preparation must be stored at between temperatures between 2° and 8° and for long term storage a temperature of -20° .The vaccine may be protected from light. At higher temperature vaccines deteriorate rapidly. For frozen vaccines, the storage temperature should be maintained as stated by the manufacturer.

LABELLING

The label or the product insert states (1) That the preparation is for Veterinary use (2) for liquid vaccines, the total number of ml in the container and for freeze dried vaccines, the number of doses in the container; (3) unless otherwise indicated the minimum number of units per dose or per ml and for the live viral vaccines, the minimum viral titre; (4) the dose and route of administration; (5) the name and proportion of any antibacterial preservative for other auxiliary substances added to the vaccine; (6) the date after which the vaccine is not intended to be used (expiry date); (7) the conditions under which it should be stored; (8) for freeze dried vaccine, the liquid to be used for the reconstitution and its volume; (9) that the vaccine should be used immediately after reconstitution or as directed in the product insert; (10) unless otherwise directed, that the vaccine should be shaken well before use; (11) species for which it is recommended (12) for oil adjuvant vaccines, that if the vaccine has been accidentally injected into humans, urgent medical attention is necessary (13) any contraindications for usage

Condition of housing of animals

1. The animals used in the production of vaccine must be housed in hygienic conditions in premises satisfactory for purpose.
2. Only healthy animals may be used in the production of vaccines. Each animal intended to be used as a source of vaccine must, before being passed for the production of vaccine be subjected to period of observation in quarantine for at least seven days. During the period of quarantine, the animal must remain free from any sign of disease and must be well kept.
3. The poultry birds from which eggs and cell culture for production of vaccines are obtained should be housed in a manner so as to keep them from extraneous infection and shall be screened at frequent intervals for common bacterial, mycoplasma and viral infections. The record of tests and their results shall be maintained by the manufacturers.

4. The animal facility having animals either for production of the vaccines or for testing should comply to CCSEA guidelines.