

# Veterinary Vaccines

## General Requirements

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, fungi or 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 antimicrobial 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 which must contain an antimicrobial preservative.

## Bacterial vaccine

Bacterial vaccines are made from inactivated or live bacteria or their antigenic components; they are liquid preparation or may be freeze dried. 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 vaccines from different species or strains. Bacterial vaccines may be prepared from cultures grown on suitable solid or liquid media.

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) Living bacterial vaccines

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

**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.

**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 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 aluminum phosphate, aluminum 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.

### **Viral Vaccine**

Viral vaccines, live or inactivated, 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 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 from the blood or tissues of a suitable host in which it has been grown *in vivo* or from tissue culture.

The seed virus used in the preparation of vaccine shall, before being used for preparing a batch, be thoroughly tested for purity, safety, sterility and antigenicity 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 stock seed virus shall be maintained by seed-lot system at specified passage level and tested for bacterial, mycoplasmal and extraneous viral contamination.

**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” unless the Pharmacopoeia otherwise provides, or if there is no other special provision in the Pharmacopoeia, some other name as approved by the licensing authority. The proper names of the vaccines included in the Pharmacopoeia are as follows:

1. Avian Infectious Bronchitis Vaccine, Inactivated
2. Avian Infectious Bronchitis Vaccine, Live
3. Avian Infectious Laryngotracheitis Vaccine, Live
4. Blue Tongue Vaccine, Inactivated
5. Canine Adenovirus Vaccine, Inactivated
6. Canine Adenovirus Vaccine, Live
7. Canine Contagious Hepatitis Vaccine, Inactivated
8. Canine Contagious Hepatitis Vaccine, Live
9. Canine Corona Virus Vaccine, Inactivated
10. Canine Distemper Vaccine, Live
11. Canine Parainfluenza Virus Vaccine, Live
12. Canine Parvovirus Vaccine, Inactivated
13. Canine Contagious Hepatitis Vaccine, Live
14. Duck Plague Vaccine, Live
15. Egg Drop Syndrome 76 (Adenovirus) Vaccine, Inactivated
16. Foot-and-Mouth Disease Vaccine, Inactivated
17. Fowl Pox vaccine, Live
18. Goat Pox Vaccine, Live
19. Inclusion Body Hepatitis (IBH) Vaccine, Inactivated
20. Infectious Avian Encephalomyelitis Vaccine, Inactivated
21. Infectious Avian Encephalomyelitis Vaccine, Live
22. Infectious Bursal Disease Vaccine, Inactivated
23. Infectious Bursal Disease Vaccine, Live
24. Infectious Chicken Anemia Vaccine, Inactivated
25. Infectious Chicken Anemia Vaccine, Live
26. Peste Des Petits Ruminants Vaccine, Live
27. Rabies Veterinary Vaccine Inactivated (Cell Culture)
28. Ranikhet Disease Vaccine, Inactivated
29. Ranikhet Disease Vaccine, Live (Lentogenic Strain)

30. Ranikhet Disease Vaccine, Live (Mesogenic Strain)
31. Reo Virus Vaccine, Inactivated
32. Reo Virus Vaccine, Live
33. **Rinderpest Vaccine, Live (delete)**
34. Sheep Pox Vaccine, Live (Attenuated)
35. Classical Swine Fever Vaccine, Live

**General standards.** Following tests are given:

- a) Description
- b) Identification
- c) Tests for sterility
- d) Purity tests for living bacterial vaccine
- e) Safety test
- f) Potency test

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.

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, mycoplasmal and viral infections. The record of tests and their results shall be maintained by the manufacturers. Should comply to Appendix XI of Drugs and Cosmetics Act, 1940 and CPCSEA guidelines.

### **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.

### **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. 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 number 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 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 and working seed 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.

## Batch Tests

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

**Aluminium** (2.3.9). Where an aluminum adsorbent has been used in the vaccine, not more than 1.25mg of aluminium (Al) per single dose, unless otherwise stated.

**Calcium** (2.3.11). Where a calcium adsorbent has been used in the vaccines, not more than 1.3mg of Calcium (Ca) per single dose, unless otherwise stated.

## Inactivating agents

**Formaldehyde** (2.3.20). Where formaldehyde has been used in the preparation of the vaccine, not more than 0.2 g/l of free formaldehyde is present in the final product, unless otherwise stated.

**Phenol** (2.3.36). Where phenol has been used in the preparation of vaccines, not more than 2.5 g/l is present in the final product, unless otherwise stated.

**Test for purity for living bacterial vaccines:** Petri-dishes containing suitable media are streaked with the final product and incubated at 37°C for 72 hours. The vaccine passes the test if no growth of micro-organisms other than those from which the vaccine was prepared is observed. Other tests include examination for motility of the organisms, fermentation reactions and agglutination test and dye-inhibitor tests in case of brucella vaccine.

**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 issue.

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

**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% 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.

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

**Pyrogens.** 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 test for pyrogens (2.2.8), unless the test for bacterial endotoxins (2.2.3), is prescribed.

**Thiomersal** (2.2.12) (2.3.48). Where thiomersal has been used in the preparation of the vaccine, not more than 0.02 per cent w/v.

**Dyes.** Approved dye may be used in sterile diluents for monitoring non-parenteral vaccination procedures. 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 than 150cm<sup>2</sup> of cell culture monolayer is inoculated with 1.0ml of inactivated harvest. The product complies with the test, if no evidence / presence of live virus or other micro-organisms is observed.

**Test for Absence of Avian Mycoplasmas** (2.7.9 ). The master seed lot complies with the test for Mycoplasmas (culture method and indicator cell culture method or Nucleic acid Amplification Test (NAT)).

**Comment from Dr. Kilari:** Why “Test for Absence of Avian Mycoplasmas” is here? It was included in viral vaccine and not essential for batch testing of other vaccines.

### **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 include.

- 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 vaccines comply with the following test. 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 2ml of the vaccine subcutaneously 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 of the second group dies or show signs of ill health. This test may be omitted if a safety test is carried out on animal of the species for which the vaccine is intended.

**Comment from Dr. Kilari:** Abnormal toxicity test can be removed as like EP if all the common guidelines of Vet vaccine development is followed (Ref: EP 8<sup>th</sup> edition, EP 7<sup>th</sup> edition supplement 7.2 and Procedia in vaccinology 5 (2011) 236-247.

**Sterility** (2.2.11). Unless otherwise stated in the individual monograph, use method A. Incubate the media for not less than 14 days at 30<sup>o</sup> to 35<sup>o</sup> in the test for detecting bacteria and at 20 to 25<sup>o</sup> in the test for detecting fungi. However, for live bacterial vaccines growth of the organisms from which the vaccine was prepared is permitted. 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 Test.** Unless otherwise stated in the individual monograph, vaccines other than live viral vaccines intended for poultry comply with following test.

Inject at least 2 healthy, susceptible animals of one of the species in which the vaccine is intended to be used by the route recommended by the manufacturer for field use. The quantity to be injected in each animal is twice the appropriate vaccinating dose. Observe the animals for not less than 7 days. No animal exhibits an abnormal reaction.

**Comment from Dr. Kilari:** There should not be any batch safety test if safety was elaborately tested during the development of vaccine.

## **Potency**

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 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.

**Antimicrobial preservative.** A suitable antimicrobial preservative may be included in sterile and inactivated vaccines and is invariably added if these preparations are issued in multidose containers, unless otherwise stated. If an antimicrobial preservative is used, it shall be shown that it does not impair the safety or efficacy of the vaccine and its effectiveness throughout the period of validity shall be demonstrated.

**Adjuvant:** Substance that is intended to enhance immune response by the vaccine.

**Stability.** Maintenance of potency of the final lot throughout the period of validity shall be demonstrated by validation studies; the loss of potency in the recommended storage conditions is assessed and excessive loss even within the limits of acceptable potency may indicate that the vaccine is unacceptable. Comply to Appendix IX Schedule Y of the Drugs & Cosmetics Act,1940.

**Labelling:** The label states (1) for liquid vaccines, the total number of ml in the container and for freeze dried vaccines, the number of doses in the container; (2) unless otherwise indicated the minimum number of units per dose or per ml of, for viral vaccines, the minimum viral titre; (3) the dose and route of administration; (4) the name and proportion of any antibacterial preservative for other auxiliary substances added to the vaccine; (5) the date after which the vaccine is not intended to be used; (6) the conditions under which it should be stored; (7) for freeze dried vaccine, the liquid to be used for the reconstitution and its volume; (8) that the vaccine should be used immediately after reconstitution; (9) unless otherwise directed. That the vaccine should be shaken well before use; (10) any contraindication to the use of the vaccine.

Name and percentage of antimicrobial preservative contained in vaccine, If vaccine is issued for sale contains any substance other than diluents, the nature and strength of such substance.

**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 2° to 8° and for long term storage -20° or below. The vaccine may be protected from light. At higher temperature vaccines deteriorate rapidly.