CHAPTER 1.1.6.

PRINCIPLES OF VETERINARY VACCINE PRODUCTION

SUMMARY

A reliable supply of pure, safe, potent, and effective vaccines is essential for maintenance of animal health and the successful operation of animal health programmes. Immunisation of animals with high quality vaccines is the primary means of control for many animal diseases. In other cases, vaccines are used in conjunction with national disease control or eradication programmes.

The requirements and procedures described here are intended to be general in nature and to be consistent with published standards that are generally available for guidance in the production of veterinary vaccines. The approach to ensuring the purity, safety, potency, and efficacy of veterinary vaccines may vary from country to country depending on local needs. However, proper standards and production controls are essential to ensure the availability of consistent, high quality products for use in animal health programmes.

As the pathogenesis and epidemiology of each disease varies, the role and efficacy of vaccination as a means of control also varies from one disease to another. Some vaccines may be highly efficacious, inducing an immunity that not only prevents clinical signs of the disease, but may also prevent infection and reduce multiplication and shedding of the disease-causing agent. Other vaccines may prevent clinical disease, but not prevent infection and/or the development of the carrier state. In other cases, immunisation may be completely ineffective or only able to reduce the severity of the disease. Thus the decision whether to recommend vaccination as part of an animal disease control strategy requires a thorough knowledge of the characteristics of the disease agent and its epidemiology, as well as the characteristics and capabilities of the various available vaccines. There is also growing public interest in the beneficial implications for animal welfare of the use of veterinary vaccines as a means of disease control. In any case, if vaccines are used, successful performance requires that they be produced in a manner that ensures a uniform and consistent product of high quality.

NOMENCLATURE

The nomenclature for veterinary biological products varies from country to country. For example, in the United States of America (USA) the term ‘vaccine’ is used for products containing live or inactivated viruses or protozoa, live bacteria, or nucleic acids. Products containing killed bacteria and other microorganisms are identified as bacterins, bacterial extracts, conventional or recombinant subunits, bacterintoxoids, or toxoids, depending on the type of antigen they contain. For example, products containing antigenic or immunising components of microorganisms may be called ‘subunits’ or ‘bacterial extracts’, and those produced from the inactivation of toxins are called ‘toxoids’. In the European Union (EU), Immunological Veterinary Medicinal Products are defined as ‘products administered to animals in order to produce active or passive immunity or to diagnose the state of immunity’, see Directive 2001/82/EC, as amended by Directive 2004/28/EC. For this chapter, however, the term ‘vaccine’ will include all products designed to stimulate active immunisation of animals against disease, without regard to the type of microorganism or microbial toxin from which they may be derived or that they contain. This use is more consistent with international nomenclature. ‘Vaccine’ will not be used in this discussion in reference to biological products recommended for passive immunisation, immunomodulation, treatment of allergies, or diagnosis.

VACCINE TYPES OR FORMS

Vaccines may be prepared as live or inactivated (killed) products. Some live vaccines are prepared from low virulence, mild, field isolates of a disease-causing agent that have been found to be safe and effective when
administered by an unnatural route or under other conditions where exposure to the microorganism will immunise rather than cause disease. Other live vaccines are prepared from isolates of disease-causing agents that have been modified by passage through laboratory animals, culture media, cell cultures, or avian embryos to select a variant of reduced virulence. The development of recombinant DNA (rDNA) procedures has provided some unique opportunities for vaccine production. Modified live vaccines may now be specifically produced by deletion of virulence-related genes from a microorganism. Others are produced by the insertion of genes that code for specific immunising antigens from a disease-causing microorganism into a nonvirulent vector microorganism. Nucleic-acid-mediated vaccines containing plasmid DNA are being developed. The DNA is usually in plasmid form and codes for immunising antigens from disease-causing microorganisms.

Killed products may contain: 1) Cultures of microorganisms that have been inactivated by chemical or other means; 2) Inactivated toxins; or 3) Subunits (antigenic parts of microorganisms) that have been extracted from cultures or that have been produced through rDNA procedures.

Both live and inactivated vaccines may be formulated with adjuvants designed to enhance their efficacy. Frequently used adjuvants are typically water-in-oil emulsions (either single or double), made with mineral or vegetable oil and an emulsifying agent. Other adjuvants, such as aluminium hydroxide gel or saponin, are also used. In addition to these traditional adjuvants, vaccines are being developed that include additional ingredients that induce immunomodulatory effects in the host animal and serve to enhance the efficacy of the product. These ingredients may include immunogenic components of microorganisms such as killed bacteria, which stimulate the immune response to other fractions contained in the vaccine, or cytokines, which may be used to regulate specific aspects of the immune system and are included in rDNA constructs used in products manufactured through biotechnology.

QUALITY ASSURANCE

The consistent production of pure, safe, potent, and efficacious vaccines requires quality assurance procedures to ensure the uniformity and consistency of the production process. As production processes for vaccines provide a great opportunity for variability, care must be taken to control variability to the greatest extent possible, preferably using validated procedures, and to protect the product from contamination through all stages of production.

Vaccine purity, safety, potency, and efficacy must be ensured by consistency in the production process. Consistent product quality (batch-to-batch uniformity) must be built in at each stage. Final product testing is used as a check to verify that the controls on the production procedures have remained intact and that the released product meets the specification previously agreed with the licensing authority.

Regulatory authorities in different countries have developed various approaches to ensuring the quality of vaccines. Although alike in their ultimate goal, these systems may vary in the emphasis given to control of the production process (process standards) in comparison with control through testing of the final product (performance standards). The control procedures selected should be those that best fit the conditions under which vaccines are being produced and, where possible, comply with good manufacturing practice.

The control standards and procedures established for a product define the risk or possibility of producing and releasing a product that is worthless, contaminated, dangerous, or harmful. The acceptable degree of risk may depend on the benefits to be gained by having the product available to prevent disease losses. Thus standards may justifiably vary from country to country or product to product, depending on local animal health conditions. However, control authorities should strive to establish control standards and procedures that ensure a finished product of the highest purity, safety, potency, and efficacy possible.

The optimal quality assurance system should address both production procedures and final product testing in proper balance. An absolutely fail-safe system that would result in no risk of releasing an unsatisfactory product would probably be too expensive with regard to cost of production as well as control. Thus regulatory officials and manufacturers of vaccines must select control procedures that are capable of ensuring an acceptable low level of risk in relation to hazard. Such procedures, however, must not be burdensome to the extent that they inhibit the development and availability of the products needed to provide proper preventative medical care at a cost that is acceptable to the consumer.

PRODUCTION FACILITIES

Facilities used for the production of vaccines should be designed to protect the purity of the product throughout the production process and to safeguard the health of the personnel. They must be constructed so that: 1) they can be readily and thoroughly cleaned; 2) they provide adequate separation of preparation rooms; 3) they have adequate ventilation; 4) they have ample clean hot and cold water and efficient drainage and plumbing; and 5) they have dressing rooms and other facilities for personnel that are accessible without passing through
biological product preparation areas. Facilities must be adequate to provide for all applicable production functions, such as: storage of master seeds, ingredients, and other production materials; preparation of growth media and cell cultures; preparation of glassware and production equipment; inoculation, incubation, and harvest of cultures; storage of in-process materials; inactivation, centrifugation, addition of adjuvant, and formulation of product; filling, desiccation, sealing of containers, labelling and storage of final product; quality control testing of in-process materials and final product; and research and development.

Separate areas are generally required for different activities. All rooms and air-handling systems must be constructed so as to prevent cross-contamination from other products and to prevent contamination by people or equipment. Virulent or dangerous microorganisms must be prepared and stored in rooms separate from the remainder of the establishment. In particular, challenge organisms must be completely separated from vaccine strains. All equipment that comes into contact with product must be sterilised using validated procedures.

Production facilities have to be designed in such a way that contamination of the external environment is prevented. Any material used during production has to be made safe before leaving the facility. If highly contagious microorganisms are propagated, the exhaust air must be treated to prevent escape of infectious agents. Personnel must follow safety procedures such as showering, and avoid contact with susceptible animals after leaving the production facilities.

Although the quality and design of production facilities may vary significantly, they must always meet standards considered to be appropriate for the vaccines that are to be produced. For example, the requirements for facilities for the production of chicken embryo vaccines administered by oral, intranasal or intraocular routes in chickens may not need to be quite as demanding as those for the production of cell culture vaccines administered subcutaneously or intramuscularly.

FACILITIES PLAN

For each vaccine made in a facility, there should be a detailed production plan that describes where each step in the production process will occur. This plan should be documented in a detailed standard operating procedure (SOP) or by providing a building blueprint and accompanying blueprint legend. Each room in the establishment should be uniquely identified, and all functions performed and all microorganisms involved should be specified for each room. Disinfection procedures, monitoring of equipment and other procedures used in the operation of the facilities to prevent contamination or errors during production should also be documented. This plan should be updated as new products or microorganisms are added to the facility, or other changes or improvements in procedures are developed.

DOCUMENTATION OF THE MANUFACTURING PROCESS

A detailed Outline of Production, a series of SOPs, or other documents should also be prepared to describe the protocol for the manufacture and testing of each product produced in an establishment. Criteria and standards for source materials should be clearly and accurately documented. Documentation should also address such things as: the source, isolation, and passage (subculturing) history of each strain of microorganism; the source and sequence of nucleic acid elements, or peptides included in products derived from biotechnology, including plasmids or other vectors used in the construction of genetically modified microorganisms for use as master seeds; methods for identifying the microorganisms and determining their virulence and purity; the medium or cell culture system used for seed and production cultures, including the methods used to demonstrate that media are free from contamination; the source of ingredients of animal origin; methods of media sterilisation; storage conditions of cell lines and seed cultures; size and types of containers used for growth of cultures; methods for preparing seed cultures and inoculating production cultures; time and conditions for incubation; observations during growth; criteria and specifications for satisfactory harvest material; and harvest techniques. There should be documentation on measures implemented by the firm to minimise the risk of transmissible spongiform encephalopathies (TSE) agent contamination in ingredients of animal origin and procedures to insure that fetal bovine serum is free of pestiviruses. It should also include: a description of all tests conducted to assess the purity and quality of the product as it proceeds through the production process; each step in the formulation of the final product; the tests used for assessing the purity, safety, potency, and other requirements of each batch/serial of completed product; the specifications for finishing, including packaging and labelling with complete indications and recommendations for use; and the expiry date established for the product.

Guidelines for the preparation of such documents for veterinary vaccines are published by competent control authorities. This documentation is intended to define the product and to establish its specifications and standards. It should serve along with the blueprints and blueprint legends (or production plan and SOPs) as a uniform and consistent method of producing the product that should be followed in the preparation of each batch/serial.
RECORD KEEPING

The producer should establish a detailed record-keeping system capable of tracking the performance of successive steps in the preparation of each biological product. Records kept should indicate the date that each essential step was taken, the name of the person who carried out the task, the identity and quantity of ingredients added or removed at each step, and any loss or gain in quantity in the course of the preparation. Records should be maintained of all tests conducted on each batch/serial. All records relevant to a batch/serial of product should be retained for at least 2 years after the expiry date on the label, or in line with the requirements of the competent control authority. In addition, a record should be maintained of all labels used on all products, with each label identified as to its name, product number, product licence number, package size, and label identification number. All labels printed should be accounted for. Records must be kept concerning sterilisation and pasteurisation procedures. These are usually made by means of automatic recording devices. The manufacturer must also keep complete records for all animals at the establishment, including health prior to being used for any tests, results of tests performed, treatment administered, maintenance, necropsy, and disposal.

MASTER SEED

The objective of testing the master seed is to ensure vaccine safety, quality and efficacy. Safety should be tested in an early stage. A master seed should be established for each microorganism used in the production of a product to serve as the source of seed for inoculation of all production cultures. Working seeds and production seeds may be prepared from the master seed by subculturing; generally the final production cultures should not be more than five (sometimes ten) passages from the master seed. The number of passages should be determined by data and designated in each case. Using a master seed and limiting the number of passages of seed microorganism in this manner assists in maintaining uniformity and consistency in production. Records of the source of the master seed should be maintained. For genetically modified microorganisms, the source of the gene(s) for the immunogenic antigens and the vector microorganism should be identified. Furthermore, the gene sequences introduced into the seed microorganism genome during construction of the modified seed should be provided. The master seed should consist of a single uniform batch/serial of seed that has been mixed and filled into containers as one batch/serial. Master seed should be frozen or desiccated and stored at low temperatures such as –40°C or –70°C, or under other conditions found to be optimal for maintaining viability. Each master seed should be tested to ensure its identity, safety and efficacy. Genetically modified seeds should also be tested to ensure stability and safety of the inserted gene sequences. Purity should also be established by testing to ensure freedom from extraneous bacteria, fungi, mycoplasma, and viruses.

MASTER CELL STOCKS

When cell cultures are used to prepare a product, a master cell stock (MCS) should be established for each type of cell to be used. Records of the source of the master cell stock should be maintained. For each product, the highest and lowest passage levels of cells that may be used for production should be established and specified in the Outline of Production or SOP. Some control authorities do not permit more than 20–40 subcultivations. Each MCS should be characterised to ensure its identity, and its genetic stability should be demonstrated when subcultured from the lowest to the highest passage used for production. The karyotype of the MCS should be shown to be stable with a low level of polyploidy. Freedom from oncogenicity or tumorogenicity should be demonstrated by in-vivo studies in appropriate species using the highest cell passage that may be used for production. Purity of MCSs should be established by testing to ensure freedom from extraneous bacteria, fungi, mycoplasma, and viruses.

• Primary cells

Primary cells are defined as a pool of original cells derived from normal tissue up to and including the tenth subculture used in the production of biologicals. In the case of products for use in poultry, these cells are usually obtained from specific pathogen free embryonating chicken eggs that have originated in an unvaccinated flock subjected to intensive microbiological monitoring. Other primary cells are derived from normal tissue of healthy animals and are tested for contamination with a wide variety of microorganisms as appropriate, including bacteria, fungi, mycoplasmas, and cytopathic and/or haemadsorbing-inducing agents or other extraneous viruses. The use of primary cells has an inherently higher risk of introducing extraneous agents compared with the use of cell lines and should be avoided where alternative methods of producing effective vaccines exist. Indeed, some control authorities only allow the use of primary cells in exceptional cases.

• Embryonating eggs

Embyronating eggs are also commonly used in the production of biologicals. In almost all cases they should be derived from specific pathogen free chicken flocks that have been intensively monitored for infectious agents and
have not been vaccinated. The route of inoculation of the egg and the choice of egg material to be harvested are dependent on the particular organism that is being propagated.

**INGREDIENTS**

The specifications and source of all product ingredients should be defined in the Outline of Production, SOP, or other appropriate documents. The Outline of Production must be approved by the National licensing agency. All ingredients of animal origin that are not subject to a validated sterilisation procedure should also be tested to ensure freedom from extraneous bacteria, fungi, mycoplasma, and viruses. Their country of origin should be known. Measures should be implemented by the firm to avoid the risk of TSE agent contamination by ingredients of animal origin. Some control authorities discourage the use of preservatives or (more importantly) antibiotics as a means of controlling adventitious contamination during production and prefer the use of strict aseptic techniques to ensure purity. However, they sometimes allow the use of preservatives in multidose containers to protect the product during use. These control authorities usually limit any addition of antibiotics in the manufacture of the product to cell culture fluids and other media, egg inocula, and material harvested from skin or possibly other tissues. They normally permit the use of no more than three antibiotics in the same product. Some control authorities prohibit the use of penicillin or streptomycin in vaccines administered by aerosol or parenterally. If the antibiotics used are not recommended for use in the target species, they should be shown to have no harmful effects in the vaccinated animals and not result in the contamination of food derived from vaccinated animals.

**SAFETY TESTS**

The intrinsic safety of vaccines should be demonstrated early in the development stage and documented as part of the licensing dossier. Safety studies during development and licensing for all products should include the safety of a single dose, of an overdose and of repeated single doses. Additional data are derived for live vaccines from the increase in virulence tests and by assessing risk to the environment and in-contact animals, as discussed below. Safety should be demonstrated in each species for which the product is indicated. As a general rule, overdose studies are required for all vaccines: ×10 for live and ×2 for inactivated vaccines (if this is not practical, an indication of safety may be obtained from the results of the potency tests). For inactivated virus or bacterial products, where host animals are used for potency testing, safety may be determined by measuring local and systemic responses following vaccination and before challenge in the potency tests. Further evidence concerning the safety of products is derived from field safety trials (discussed below). Vaccines derived through biotechnology should be evaluated as discussed in the classification of biotechnology-derived vaccines and release of live rDNA vaccines below.

**INCREASE IN VIRULENCE TESTS**

With live vaccines, there is concern that the organism might be shed from the host and transmitted to contact animals, causing disease if it retains residual virulence or reverts to virulence. Therefore, all live vaccines should be tested for virulence by means of passage studies. Vaccine organisms are propagated in vivo by inoculating a group of target animals with master seed, in principle; this inoculation uses the natural route of infection for that organism that is most likely to result in infection and reversion and, if possible, that represents a recommended route of administration of the vaccine manufactured from this master seed. The vaccine organism is recovered from tissues or excretions and is used directly to inoculate a further group of animals, and so on. After not less than four passages, i.e. use of a total of five groups of animals (more for poultry products), the isolate must be fully characterised, using the same procedures used to characterise the master seed. Regulatory authorities opinion varies in whether or not it is acceptable to propagate in vitro between passages organisms that otherwise cannot be passaged five times because of their degree of attenuation. The vaccine organism must retain an acceptable level of attenuation after propagation in this way.

**ASSESSING RISK TO THE ENVIRONMENT**

The ability of each live vaccine to shed, to spread to contact target and non-target animals, and to persist in the environment must be evaluated to provide information for assessing the risk of the vaccine to the environment, taking into account human health. In some cases this may be done in conjunction with the increase in virulence tests. These and additional considerations are especially important in the case of products based on biotechnology or recombinant DNA techniques; more information about such products is provided in the sections at the end of this chapter.
EFFICACY TESTS

The efficacy of veterinary vaccines should be demonstrated by statistically valid vaccination–challenge studies in the host animal, using the most sensitive, usually the youngest, animals for which the product is to be recommended. Data should support the efficacy of the vaccine in each animal species by each vaccination regimen that is described in the product label recommendation, including studies on the onset of protection when claims for onset are made in the product labelling and for the duration of immunity. The tests should be performed under controlled conditions starting, wherever possible, with seronegative animals. Where validated potency tests are available, target species vaccination–challenge studies may not be required if predictive serological test results are available. The application of procedures to replace, reduce, and refine animal tests (the ‘three Rs rule’) should be encouraged whenever possible.

Efficacy studies should be conducted with final product vaccine that has been produced at the highest passage level from the master seed that is permitted in the Outline of Production, or other documentation of the manufacturing process. This will have specified the minimum amount of antigen per dose that must be in the final product throughout the entire authorised shelf-life. Where a range of antigen level per dose is permitted, the antigen level per dose in the vaccine tested for efficacy must be at or below the minimum permitted amount. The precise challenge method and the criteria for determining protection vary with the immunising agent and should be standardised whenever possible.

Field efficacy studies may be used to confirm the results of laboratory studies or to demonstrate efficacy when meaningful vaccination–challenge studies are not feasible. However, it is generally more difficult to obtain statistically significant data to demonstrate efficacy under field conditions. Protocols for field studies are more complex, and care must be given to establish proper controls to ensure the validity of the data. Even when properly designed, field efficacy studies may be inconclusive because of uncontrollable outside influences. Some problems include: a highly variable level of challenge; a low incidence of disease in nonvaccinated controls; and exposure to other organisms causing a similar disease. Therefore, efficacy data from both laboratory and field studies may be required to establish the efficacy of some products, as well as ‘posteriory’ field trials linked to vaccinovigilance.

INTERFERENCE TESTS

For products with two or more antigenic components, tests must confirm that there is no interference between individual components, that is, one component causing a decrease in the protective immunological response to another component. Interference testing should be conducted for each combination product prior to approval.

A loss of potency may also result when residual inactivating agent in a killed liquid product used as a diluent for a desiccated live fraction reduces the viability of the live organisms because of viricidal or bacteriocidal activity. Each batch/serial of liquid killed vaccine that is to be used as a diluent for live vaccines must, therefore, be tested for viricidal or bacteriocidal activity prior to release.

Consideration must also be given to possible interference between two different vaccines from the same manufacturer recommended to be given to the same animal within a 2-week period.

CONSISTENCY OF PRODUCTION

Prior to marketing approval of any new product, each establishment should produce in its facilities three consecutive production batches/serials of completed product to evaluate the consistency of production. These batches/serials should be prepared according to the procedures described in the Outline of Production and blueprints and legends, SOPs or other documentation of the manufacturing process and should therefore be ‘typical of production’. Some authorities require that the size of each of the three batches/serials should be at least one-third the size of the average batch/serial that will be produced once the product is in production.

The manufacturer should test each of these batches/serials for purity, safety, and potency as provided in the Outline of Production or other documentation of the manufacturing process. Applicable Standard Requirements and test procedures, for example those described in CFR (Code of Federal Regulations) Title 9 part 113, in the Annex to EU Directive 2001/82/EC (as amended), in the European Pharmacopoeia, or as described in this Terrestrial Manual may be used. Satisfactory test results should be demonstrated for all three batches/serials prior to approving the production of the product in the facilities and its release for marketing. Each subsequent batch/serial should be tested in the same manner with satisfactory results prior to release for marketing.
STABILITY TESTS

Stability studies (based on an acceptable potency test) are required to establish the validity of the expiry date that appears on the product package. Some authorities allow the use of accelerated stability tests to determine a provisional expiry date for products, e.g., incubating at 37°C for 1 week for each year of dating. Such estimates must be confirmed by periodic real-time potency tests on at least three different batches/serials through the period of time indicated by the expiry date, and 3–6 months beyond. For products containing viable organisms, testing should be done at release and at the approximate expiry date until a statistically valid record has been established. For non-viable products, each batch/serial presented for licensing is tested at release and at periodic intervals through, or past, the requested expiry date. If at the end of the dating period (shelf life) specified, the product is tested and found still to be above the release quality, consideration can be given to extending the designated shelf life, by request to the control authority. Stability testing also provides the opportunity to test for residual moisture and for other important parameters, such as the stability of adjuvant emulsions.

BATCH/SERIAL RELEASE FOR DISTRIBUTION

Prior to release, the manufacturer must test each batch/serial for purity, safety, and potency, as well as perform any other tests described in the firm’s Outline of Production or other documentation of the manufacturing process for that product. In countries that have national regulatory programmes that include official control authority re-testing (check testing) of final products, samples of each batch/serial should also be submitted for testing in government laboratories by competent authorities. If unsatisfactory results are obtained for tests conducted either by the manufacturer or by competent authorities, the batch/serial should not be released. In such cases, subsequent batches/serials of the product should be given priority for check testing by competent authorities.

1. Batch/serial purity test

Purity is determined by testing for a variety of contaminants. Tests to detect contaminants are performed on: master seeds, primary cells, MCs, ingredients of animal origin if not subjected to sterilisation (e.g. fetal bovine serum, bovine albumin, or trypsin), and each batch/serial of final product prior to release.

Purity test procedures have been published, for example in CFR Title 9 part 113, in the European Pharmacopoeia, or in this Terrestrial Manual, for the detection of extraneous viruses, bacteria, mycoplasma and fungi, including for example: Salmonella, Brucella, chlamydial agents, haemagglutinating viruses, avian lymphoid leukemia, pathogens detected by a chicken inoculation test, chicken embryo inoculation test, lymphocytic choriomeningitis, cytopathic and haemadsorbing agents, and pathogens detected by enzyme-linked immunosorbent assay, polymerase chain reaction, or the fluorescent antibody technique. Procedures used to ensure that fetal or calf serum and other ingredients of bovine origin are free of pestiviruses should be of high concern and well documented. Tests to be used to ensure purity vary with the nature of the product, and should be prescribed in the Outline of Production or other documentation of the manufacturing process. As tests for the detection of TSE agents in ingredients of animal origin have not been developed, vaccine manufacturers should document in their Outlines of Production or SOPs the measures they have implemented to minimise the risk of such contamination in ingredients of animal origin. This relies on three principles: first, verification that sources of all ingredients of animal origin in production facilities are from countries recognised as having the lowest possible risk of bovine spongiform encephalopathy; second, that the tissues or other substances used are themselves recognised as being of low or nil risk of containing TSE agents; third, where relevant, that the processes applied to the material have been validated for inactivation of TSE agents. Methods of production should also document the measures taken to prevent cross contamination of low risk materials by higher risk materials during processing.

2. Batch/serial safety test

Batch/serial safety tests are required for the release of each batch/serial and typical tests are described in CFR Title 9 part 113, in the European Pharmacopoeia, in this Terrestrial Manual and elsewhere. Standard procedures are given for safety tests in mice, guinea-pigs, cats, dogs, horses, pigs, and sheep and are generally conducted using fewer animals than are used in the safety tests required for licensing. Batches/serials are considered satisfactory if local and systemic reactions to vaccination with the batch/serial to be released are in line with those described in the registration dossier and product literature. Some authorities do not permit batch/serial safety testing in laboratory animals, requiring a test in one of the target species for the product.

3. Batch/serial potency test

Batch/serial potency tests, required for each batch/serial prior to release, are designed to correlate with the host animal vaccination–challenge efficacy studies. For inactivated viral or bacterial products, potency tests may be
conducted in laboratory or host animals, or by means of quantitative in-vitro methods that have been validated reliably to correlate in vitro quantification of important antigen(s) with in vivo efficacy. The potency of live vaccines is generally measured by means of bacterial counts or virus titration. Recombinant DNA or biotechnology-based vaccines should also be tested. Live genetically modified organisms can be quantified like any other live vaccine by titration, and expressed products of recombinant technology are quantified by in vitro tests, which can be easier to perform compared with tests on naturally grown antigens because of the in-process purification of the desired product.

When testing a live bacterial vaccine for release for marketing, the bacterial count must be sufficiently greater than that shown to be protective in the master seed immunogenicity (efficacy) test to ensure that at any time prior to the expiry date, the count will be at least equal to that used in the immunogenicity test. When testing a live viral vaccine for release, the virus titre must, as a rule, be sufficiently greater than that shown to be protective in the master seed immunogenicity test in order to ensure that at any time prior to the expiry date, the titre will be at least equal to that used in the immunogenicity test. Some control authorities specify higher bacterial or viral content than these. It is evident that the appropriate release titre is primarily dependent on the required potency and secondarily dependent on the rate of decay of the bacteria or viruses in the vaccine, as indicated by the stability test.

Standard Requirements have been developed and published by competent authorities for potency testing several vaccines. These tests can be found in CFR Title 9 part 113, in the European Pharmacopoeia, and in this Terrestrial Manual.

OTHER TESTS

Depending on the form of vaccine being produced, certain tests may be indicated and should be provided as appropriate in the Outline of Production or other documentation of the manufacturing process. These tests may concern: the level of moisture contained in desiccated products, the level of residual inactivant in killed products, the complete inactivation of killed products, pH, the level of preservatives and permitted antibiotics, physical stability of adjuvants, retention of vacuum in desiccated products, and a general physical examination of the final vaccine. Tests for these purposes may also be found in CFR Title 9 part 113, in EU Directive 2001/82/EC (as amended), in the European Pharmacopoeia, or in this Terrestrial Manual.

SAMPLING

Samples should be selected from each batch/serial of product. The selector should pick representative final containers from each batch/serial and store these samples at the storage temperature recommended on the label. The producer should keep these reserve samples at the recommended storage temperature for a minimum of 6 months after the expiry date shown on the label, so that they are available to assist in evaluating the cause of any field problems reported from the use of the vaccine. The samples should be stored in a secure storage area and be tamper-evident.

LABELLING

Standards for labelling products will vary from country to country; however, the label indications and all claims that are made on the label should be supported by appropriate data that have been reviewed and approved by competent authorities. It is recommended that all labels for veterinary vaccines be water-proof and contain the following information, although for very small containers, the label may instead refer to the carton label or to an enclosed package insert for some of the less prominent information:

1. The true name of the product, prominently lettered and with equal emphasis on each word;
2. The name and address of the producer (and also the importer for imported products);
3. The recommended storage temperature;
4. A statement that the product is ‘for veterinary (or animal) use only’. Full instructions for use, including all required warnings;
5. For food animals, a statement indicating that the animals should not be vaccinated within a specified number of days before slaughter. This will depend on the vaccine (e.g. type of adjuvant) and is not required for all products;
6. The expiry date;
7. The batch/serial number by which to identify the product in the producer’s record of preparation;
8. The licence number for the product; in some countries this is replaced by the licence number of the establishment/manufacturer;
9. The recoverable quantity and number of doses;
10. A statement that the entire contents of a multidose container should be used when the container is first opened (or with appropriate holding time for certain products, as supported by data) and that any unused portions should be disposed of in a proper manner;
11. A safety warning to the operator, if appropriate, e.g. accidental self-injection with oil emulsion vaccines.
12. Where it is allowed for an antibiotic to be added to a vaccine during the production process, the statement "Contains (antibiotic name) as a preservative" or an equivalent statement indicating the antibiotic added should appear on the carton or enclosures if used. If cartons are not used, such information should appear on the final container label.

Labels may also include other factual statements that are not false or misleading. Special restrictions concerning the use or handling of the product, when applicable, should also be indicated.

Similar information should also be given in a Product Data Sheet that is provided as a package insert. This will also contain much more detail about method of use and possible adverse reactions.

FIELD TESTS (SAFETY AND EFFICACY)

All veterinary biological products administered to animals should be tested for safety and, if possible, for efficacy in the field, using good clinical practice, before being authorised for general use. Field studies are designed to demonstrate efficacy under working conditions and to detect unexpected reactions, including mortality, that may not have been observed during the development of the product. Under field conditions there are many uncontrollable variables that make it difficult to obtain good efficacy data, but demonstration of safety is more reliable. The tests should be done on the host animal, at a variety of geographical locations, using appropriate numbers of susceptible animals. The test animals should represent all the ages and husbandry practices for which the product is indicated; unvaccinated controls must be included. The product tested should be one or more production batches/serials. A protocol should be developed indicating the observation methods and the recording methods.

INSPECTION OF PRODUCTION FACILITIES

Establishments that are approved to produce veterinary biologicals should be subject to in-depth inspections of the entire premises by national competent authorities to ensure compliance with the Outline of Production and blueprints and legends, SOPs, or other documentation of the manufacturing process. These inspections may include such items as: personnel qualifications; record keeping; general sanitation and laboratory standards; research activities on products being developed; production procedures; operation of sterilisers, pasteurisers, incubators, and refrigerators; filling, desiccating, and finishing procedures; care and control of animals; testing procedures; distribution and marketing; and product destruction. It is desirable to have good manufacturing practice (for manufacturing) and good laboratory practice (for quality assurance testing). (See chapter 1.1.4 Quality management in veterinary testing laboratories, for guidelines.)

The inspectors should prepare a comprehensive report documenting the findings of the inspection and stating the actions that the establishment must take to improve its production processes. The establishment should receive a copy of the report. When necessary a follow-up inspection should be conducted to determine whether appropriate action has been taken to correct deficiencies. Continued reassessment in this manner is needed to ensure that production facilities continue to be operated in an acceptable manner.

UPDATING THE OUTLINE OF PRODUCTION

Before production procedures are changed, the corresponding Outline of Production or other documentation of the manufacturing process should be changed. Establishments should have internal review procedures to evaluate all changes in production before they are initiated. Changes should also be reviewed and approved by competent authorities prior to their implementation. In cases where a significant production step is altered, revisions may require additional data to support the purity, safety, potency, and/or efficacy of the product. In
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countries with regulatory programmes that include check testing the final product at national laboratories, revisions should entail testing of the new product by competent authorities.

PERFORMANCE MONITORING

Manufacturers are required to maintain an adverse reaction notification system and an effective mechanism for rapid product recall. These should both be subject to audit by regulatory bodies. In many countries, the manufacturer must notify all adverse reactions immediately to the regulatory authority, along with any remedial action taken. An alternative used in some countries is that if at any time, there are indications that raise questions regarding the purity, safety potency, or efficacy of a product, or if it appears that there may be a problem regarding the preparation, testing or distribution of a product, the manufacturer must immediately notify the regulatory authorities concerning the circumstances and the action taken.

After release of a product, its performance under field conditions should continue to be monitored by competent authorities. Consumer complaints may serve as one source of information; however, such information needs to be investigated to determine whether or not the reported observations are related to the use of the product. Users of veterinary vaccines should be informed of the proper procedures for making their complaints. The manufacturer of the product should be informed of all complaints received by competent authorities. Competent authorities should also ascertain whether they have received other similar complaints for this product and, if so, whether the manufacturer has taken appropriate action. Control laboratories may test samples of the batch/serial of product involved, if necessary.

When the investigation is complete, a final report should be prepared and a summary of the findings sent to the complainant and to the manufacturer. When it is determined that a product is causing serious problems, immediate action should be taken to remove the product from the market and to notify animal health authorities.

ENFORCEMENT

National programmes established to ensure the purity, safety, potency, and efficacy of veterinary vaccines must have adequate legal authority to ensure compliance with product registration conditions and other programme requirements. The goal should be to obtain voluntary compliance with established regulatory requirements. However, when violations occur, competent authorities must have adequate legal authority to protect animal and human health. Authority for detention, seizure, and condemnation of products found to be worthless, contaminated, dangerous, or harmful may be valuable for this purpose. Under such authority, product may be detained for a period of time, and if during that time compliance cannot be achieved, competent authorities may seek a court order or decree for seizure and condemnation.

The authority to remove or suspend establishment and/or product licenses, obtain injunctions, and stop the sale of product is also needed. Civil penalties or criminal prosecution may also be necessary for serious or deliberate violations.

LICENSING OF PRODUCTS DERIVED THROUGH BIOTECHNOLOGY

Recent advances in biotechnology have made possible the development and commercialisation of new biological products with useful antigenic and diagnostic properties. Many such products have now been licensed or approved, and more are being developed. Products of rDNA technology do not differ fundamentally from conventional products. Therefore, existing laws and regulations are fully applicable to these new products.

CLASSIFICATION OF BIOTECHNOLOGY-DERIVED VACCINES

Each competent authority with power to regulate organisms and products derived from recombinant techniques should ensure that the public health and the environment are protected from any potentially harmful effect. For the purpose of evaluating licence applications, veterinary vaccines derived through rDNA technology may be divided into three broad categories. The division is based on the products’ biological properties and on the safety concerns they present.

Category I consists of nonviable or killed products that pose no risk to the environment and present no new or unusual safety concerns. Such products include inactivated microorganisms, either whole or as subunits, created by using rDNA techniques.
Category II products contain live microorganisms modified by adding or deleting one or more gene(s). Added genes may code for marker antigens, enzymes, or other biochemical by-products. Deleted genes may code for virulence, oncogenicity, marker antigens, enzymes, or other biochemical by-products. The licence application must include a characterisation of the DNA segments added or deleted, as well as a phenotypic characterisation of the altered organism. The genetic modifications must not result in any increase in virulence, pathogenicity, or survivability of the altered organism in comparison with the wild-type form. It is important that the genetic modification does not cause a deterioration in the safety characteristics of the organism.

Category III products make use of live vectors to carry recombinant-derived foreign genes that code for immunising antigens. Live vectors may carry one or more foreign gene(s) that have been shown to be effective for immunising target host animals. The use of DNA vaccines containing recombinant-derived foreign genes that code for immunising antigens (plasmid DNA vaccines) constitutes a new approach to vaccine development. The proper categorisation of this type of rDNA-derived product will be established as biological properties and safety characteristics are determined. These new vaccines may find application in a wide variety of situations much as conventional products have. Guidelines for the development, production, characterisation, and control of these new products are still preliminary and subject to change as new data and knowledge are developed. Information concerning the current thinking on regulatory guidelines may be found on the Internet at the following addresses:

http://www.orcbs.msu.edu/biological/biolsaf.htm; http://www.pestlaw.com/index.html;

RELEASE OF LIVE rDNA PRODUCTS

The release of live rDNA microorganisms (Categories II and III) for field testing or general distributions as an approved or licensed product may have a significant effect on the quality of the human and animal environment. Before release is authorised, the manufacturers of the vaccine should conduct a risk assessment to evaluate the impact on the human and animal environment. In the USA, for example, a procedure is adopted that could be used as a model system in other countries. The European Union has adopted a similar system. It is performed as follows:

A risk assessment is carried out that should contain the following information: the purpose and need for the proposed action; the alternatives considered; a list of the government agencies, organisations, and persons consulted; and the affected environment and the potential environmental consequences. The topics discussed should include: the characteristics of the vaccine organism, human health risks, animal health risks for both target and nontarget animals, persistence in the environment, and increase in virulence.

If the risk assessment results in a finding by competent authorities that the proposed release of the recombinant vaccine into the environment for field trials or general distribution would not have a significant impact on the environment, a notice should be published and distributed to the public announcing this and that the risk assessment and findings are available for public review and comment. If no substantive comments are received to refute the findings, competent authorities may authorise the field testing or grant the license or approval for general distribution.

The preparation of a risk assessment and the findings made from the assessment may also include the scheduling of one or more public meetings if a proposed action has ecological or public health significance. Such meetings should be announced through a public notice. Interested persons should be invited to make presentations, along with presentations by the producer of the product, and government personnel. The transcripts of such meetings should become part of the public record.

If, in the course of preparing a risk assessment, competent authorities conclude that the proposed action may have a significant effect on the human environment, an Environmental Impact Statement (EIS) should be prepared. The EIS provides a full and fair discussion of the significant environmental impacts, and informs decision-makers and the public of any reasonable alternatives that would avoid or minimise the adverse impacts. (Environmental documents are considered in CFR Title 40 part 1508.) See also EU Directive 2001/18/EC and http://www.emea.europa.eu/pdfs/vet/iwp/000404en.pdf

FURTHER READING

The following are some suggested texts that contain guidelines on aspects of vaccine production.

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USDA-APHIS-VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1995). Veterinary Biologics General Licensing Considerations No. 800.200, Efficacy Studies. USDA-APHIS-Veterinary Biologics, 4700 River Road, Riverdale, Maryland 20737, USA.


APPENDIX 1.1.6.1.

RISK ANALYSIS FOR BIOLOGICALS FOR VETERINARY USE

GENERAL CONSIDERATIONS

All products, including biologicals for veterinary use, derived from animals have some capacity to transmit animal disease. The level of this capacity depends on the inherent nature of the products, their source, the treatment that they might have undergone, and the purpose for which they are intended. Biologicals for *in vivo* use in particular will have the highest probability of exposure to animals and as such present the highest risk. Products used for *in vitro* purposes can introduce disease into animal populations through deliberate or inadvertent use *in vivo*, contamination of other biologicals, or spread by other means. Even products for diagnosis and research have the potential for close contact with animals. Exotic micro-organisms, some highly pathogenic, which may be held for research and diagnostic purposes in countries free from infection or the diseases they cause, could possibly contaminate other biological products.

Veterinary Authorities of importing countries shall make available specific procedural requirements for approval or licensing of biologicals for veterinary use. They may limit supply to registered institutions or *in vitro* use or for non-veterinary purposes where such assurance cannot be provided.

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APPENDIX 1.1.6.2.

RISK ANALYSIS FOR VETERINARY VACCINES

INTRODUCTION

Risk analysis for veterinary vaccines has to be founded on the principles of quality assurance, which includes quality control, in the production of veterinary vaccines. These recommendations are focused mainly on the risk related to the contamination of vaccines by infectious agents particularly in regard to the risk of importing exotic diseases. The major risk of introducing a disease into a country is through importation of live animals or animal products and rarely through veterinary vaccines. Veterinary vaccines can however be contaminated by disease agents if master seeds, strains, cell cultures, animals or ingredients of animal origin such as fetal calf serum used in production are contaminated or if cross contamination occurs during the production process.

PRINCIPLES

Exporting countries and importing countries should agree on a system of classification of risks associated with veterinary vaccines taking into account factors such as purification procedures which have been applied.

Exporting countries and importing countries should agree on risk analysis models to address specific issues and products. Such risk analysis models should include a scientific risk assessment and formalised procedures for making risk management recommendations and communicating risk. The regulation of veterinary vaccines should include the use of either qualitative or quantitative models.

Risk analysis should be as objective and transparent as possible. Step risk and scenario tree methods should be used in risk assessment whenever appropriate, as they identify the critical steps in the production and use of the products where risks arise and help to characterise those risks.

The same conclusions about risk analysis may be reached by differing methods. Where methods may differ in countries, the concept of equivalence should apply wherever possible and the methods should be validated to ensure they are of comparable sensitivity.

MANUFACTURING PRACTICES

The manufacture of veterinary vaccines has special characteristics which should be taken into consideration when implementing and assessing the quality assurance system. Due to the large number of animal species and related pathogenic agents, the variety of products manufactured is very wide and the volume of manufacture is often low; hence, work on a group basis is common. Moreover, because of the very nature of this manufacture (cultivation steps, lack of terminal sterilisation, etc.), the products must be particularly well protected against contamination and cross contamination. The environment must also be protected especially when the manufacture involves the use of pathogenic or exotic biological agents and the worker must be particularly well-protected when the manufacture involves the use of biological agents pathogenic to man.

These factors, together with the inherent variability of immunological products, means that the role of the quality assurance system is of the utmost importance. It is important that vaccines should be manufactured in accordance with a recognised codified system that includes specifications regarding equipment, premises, qualification of personnel as well as quality assurance and regular inspections.

A commonly agreed system of facility inspection carried out by qualified and specialised inspectors must be in place to assure confidence.
INFORMATION TO BE SUBMITTED WHEN APPLYING FOR REGISTRATION IN THE IMPORTING COUNTRY

The manufacturer or Veterinary Authority of the exporting country should make available to the importing country the pharmacopoeia it uses. For the importing country it is necessary to have documented both the quality control methods used and the source of each batch of starting materials. The key steps of the manufacturing process of veterinary vaccines should be described in detail to help risk analysis. Risk analysis has to be focused on the quality and safety parts of the application file. Laboratory safety testing should cover target and non-target organisms to obtain sufficient biological data. All test procedures used should correspond with the state of scientific knowledge at the time and should be validated.

The description of the method of preparation of the finished product should include an adequate characterisation of the substances needed to prepare the working seeds, the description of the treatments applied to starting materials to prevent contamination, and a statement of the stages of manufacture at which sampling is carried out for process control tests.

The results of control tests during production and on finished product, as well as the sensitivity of these tests, have to be available for risk analysis. The stepwise procedures of the control tests should also be available.

CATEGORISATION OF VETERINARY VACCINES

To assist in risk analysis, countries should establish a system of categorisation of veterinary vaccines taking into account criteria such as pathogens used as active ingredients, their inherent characteristics and the risk they pose.

In case of live vectored vaccines, the safety of the vector to the targeted and non-targeted species and to human beings must be assessed. Special attention should be paid to potential tissue tropism or host range modification of the recombinant.

VACCINOVIGILANCE

Exporting countries and importing countries should ensure that a reliable system of vaccinovigilance (post licensing monitoring) is established to identify, at the earliest stage, any serious problems encountered from the use of veterinary vaccines. Vaccinovigilance should be ongoing and an integral part of all regulatory programmes for veterinary vaccines, especially live vaccines.

RISK COMMUNICATION

Reliable data in support of applications submitted in importing countries should be provided by the manufacturer or the Veterinary Authority of the exporting country. Relevant data on risk analysis, changes in animal health situations and vaccinovigilance should be shared by Veterinary Authorities on a continuous basis.

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