This chapter has been extensively revised and updated. Although some portions of the existing text have been incorporated, new text and deleted text have not been marked, in the interest of clarity

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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.

As for all medicines, vaccine production starts within research and development (R&D) facilities, carrying out all the preclinical studies which are intended to demonstrate the quality of the products, including the safety and the efficacy. All these studies are generally carried out according to international reference standards such as good laboratory practice (GLP) for preclinical studies and good clinical practice (GCP) for clinical studies.

Before release of a vaccine for use in a country, a license or marketing authorisation must be requested from and be assessed and authorised by the competent authority to ensure compliance with local product marketing authorisation conditions. Starting materials to be used, manufacturing steps, in-process controls and controls on the finished product before release by a responsible person should be described in the marketing authorisation dossier, as should be the necessary tests to demonstrate quality, safety, and efficacy of the vaccine.

After the marketing authorisation has been granted by a competent authority, the industrial production can be launched in a manufacturing site which is authorised by the competent authority in accordance with national requirements and having the relevant equipment, facilities and personnel for production and controls. The manufacturing site should be inspected on a regular basis by experienced official inspectors.

Quality assurance is an integral part of the production of pure, safe and efficacious vaccines. This chapter outlines critical check points, with more details provided in chapters 1.1.8

Recommendations for manufacturing sites for veterinary vaccines *and 1.1.9* Quality control of vaccines*. It is a step-wise and iterative process. Compliance with the full standards described in these chapters can be achieved through risk analysis and step-wise process improvement.*

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[1] 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 physical 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 contain different antigenic components and may be formulated with adjuvants, stabilisers, antimicrobial preservatives and diluents. Adjuvants are designed to enhance the immunising efficacy of the vaccine. Those used frequently 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.

Many products obtained by biotechnology 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.

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. 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.

1 The generic term "live" (usually modified or attenuated) is used throughout this *Terrestrial Manual* to differentiate from inactivated organisms, although it is recognised that in the case of viruses they cannot be considered truly alive.

Category I consists of non-viable or killed products that pose negligible 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 marketing authorisation 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 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.

VACCINE PRODUCTION

1. Quality assurance

Quality assurance is a wide-ranging concept that covers all matters that individually or collectively influence the quality of a product. It is the total sum of the organised arrangements made with the object of ensuring that medicinal products are of the quality required for their intended use, ranging from process control, improvement and inspection, testing of the quality, efficacy and safety of the vaccines to assurance achieved through competent authority procedures. It is a step-wise and iterative process, and compliance with the standards described in these chapters can be achieved through risk analysis and step-wise process improvement. The basic concepts of quality assurance, good manufacturing practice (GMP), quality risk management and quality control are inter-related. See chapter 1.1.9 for full details.

2. 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, and provide secure containment of any disease causing agents.

For each vaccine, 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.

The requirements for vaccine production facilities are described in more detail in chapter 1.1.8.

3. Documentation of the manufacturing process and record keeping

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.

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 (one master batch record for each product).

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.

Details of documentation required at a manufacturing site are described in chapter 1.1.8.

4. Production

Because of the wide variety of products, the frequently large number of stages involved in the manufacture of vaccines and the nature of the biological processes, each stage must be constantly monitored. Adherence to validated operating procedures and in-process controls is critical.

The specifications and source of all product ingredients should be defined in the Outline of Production, SOPs, or other appropriate documents. The Outline of Production must be approved by the competent authority. 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 as specified in Chapter 1.1.7. *Tests for sterility and freedom from contamination of biological materials*. Their country of origin should be known. Measures should be implemented by the manufacturer to avoid the risk of transmissible spongiform encephalopathy (TSE) agent contamination by ingredients of animal origin.

Some control authorities discourage the use of preservatives, especially 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. 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.

Details of vaccines production required at a manufacturing site, including requirements for staring materials, cell bank systems and seed-lot systems are described in chapter 1.1.8.

5. Process validation

Prior to obtaining a marketing authorisation for any new product, each establishment should produce in its facilities three consecutive production batches/serials of completed product to evaluate the consistency of production. The process used should be representative of the manufacturing procedure shown to be safe and efficacious during the preclinical studies.

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 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.

6. Stability tests

It is important to monitor the stability of each product through a programme of on-going stability. Additional information is given in the chapter 1.1.9.

Conditions of storage affecting the quality of the product should be taken into account as evaluated in the marketing authorisation, including light, temperature and the adhesive/absorptive properties of containers. All vaccines are sensitive to heat to some extent, but some are more sensitive than others. There is increasing interest in the development of vaccines that can tolerate adverse storage conditions. In this *Terrestrial Manual*, thermotolerant (see Glossary of Terms) is defined as the ability of live vaccines to retain a level of infectivity after exposure to heat, that is, the delayed heat degradation temperatures above 8°C. It is defined by the length of time the vaccine will retain a potency sufficient to induce a protective immune response. By the latter criterion the term can also be applied to killed vaccines.

7. Tests to demonstrate safety and efficacy of a vaccine

All laboratory procedures and tests should be conducted in compliance with an international standard such as Good Laboratory Practice (GLP), see chapter 1.1.9. Similarly tests in animals should comply with Good Clinical Practice (GCP). Submission of the results of the tests described below would normally be required in a dossier supporting a request for the granting of a marketing authorisation or license.

7.1. Safety tests

7.1.1. Target animal safety tests

Harmonised international guidelines for safety tests are published by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products (VICH) in VICH GL 44 Target animal safety for veterinary live and inactivated vaccines (http://www.vichsec.org/guidelines/biologicals/bio-safety/target-animal-safety.html). An overdose test is required for live vaccines shown to retain residual pathogenicity by induction of disease-specific signs or lesions. In general other vaccines do not require overdose testing.

For vaccines that require a single life-time dose or primary vaccination series only, the primary vaccination regimen should be used. For vaccines that require a single dose or primary vaccination series followed by booster vaccination, the primary vaccination regimen plus an additional dose should be used.

The intrinsic safety of vaccines should be demonstrated early in product development and documented as part of the licensing dossier. Safety studies during development and licensing should include the safety of a single dose for all products, as well as the safety of an overdose in the case of live vaccines and of repeated single doses for vaccines that require more than one dose during the lifetime of the animal. 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.

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.

7.1.2. 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 with repeated host passages. Guidelines for testing are published by VICH: GL 41: Examination of live veterinary vaccines in target animals for absence of reversion to virulence (http://www.vichsec.org/guidelines/biologicals/bio-safety/target-animal-safety.html).

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 the organism that is most likely to result in infection and reversion or 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, the isolate must be fully characterised, using the same procedures used to characterise the master seed. Regulatory authority 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.

7.1.3. 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. In the case of live vaccines strains that may be zoonotic, the risk for humans should be assessed. 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 other sections.

7.2. Efficacy tests

7.2.1. Laboratory efficacy

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. This includes studies regarding 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 non-vaccinated 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 '*a posteriori'* field trials linked to vaccinovigilance.

7.2.2. Interference tests

Consideration must 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. The safety and the efficacy of this association should be investigated.

7.2.3. Field tests (safety and efficacy)

7.2.3.1. All vaccines

All veterinary vaccines administered to animals should be tested for safety and, if possible, for efficacy in the field, using GCP, 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.

7.2.3.2. Additional requirements for live rDNA products

The release of live rDNA microorganisms (Categories II and III) for field testing or general distribution 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 EU has adopted a similar system. It is performed as follows:

A risk assessment is carried out that should contain the following information:

i) the purpose and need for the proposed action;

ii) the alternatives considered;

iii) a list of the government agencies, organisations, and persons consulted;

iv) the affected environment and the potential environmental consequences.

The topics discussed should include:

i) the characteristics of the vaccine organism,

ii) human health risks,

iii) animal health risks for both target and nontarget animals,

iv) 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 the United States Code of Federal Regulations [CFR] Title 40 part 1508. The EU has issued guidelines under Directive 2001/18/EC: *Guideline on Live Recombinant Vector Vaccines for Veterinary Use,* see http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004590.pdf

8. 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, efficacy or stability of the product. In countries with regulatory systems that include confirmatory testing the final product at national laboratories, revisions should entail testing of the revised product by competent authorities.

QUALITY CONTROLS IN VACCINE PRODUCTION

1. Principle

Quality control is concerned with sampling, specifications and testing as well as the organisation, documentation and release procedures. Quality control ensures the necessary and relevant tests are carried out, and that materials and equipment are not released for use, nor products released for sale or supply, until their quality has been judged satisfactory. Quality control is not confined to laboratory operations, but must be involved in all decisions that may concern the quality of the product. The independence of quality control from production is considered fundamental to the satisfactory operation of quality control. Details of quality control are described in the chapter 1.1.9.

2. Batch/serial release for distribution

Prior to release, the manufacturer must test a representative sample of 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.

2.1. Batch/serial purity test

Purity is determined by testing for a variety of contaminants. Tests to detect contaminants are performed on a representative sample of 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 annex to EU Directive 2001/82/EC (as amended), in the European Pharmacopoeia, or in this *Terrestrial Manual* (chapter 1.1.7), for the detection of extraneous viruses, bacteria, mycoplasma and fungi. Examples include tests for: *Salmonella*, *Brucella,* chlamydial agents, haemagglutinating viruses, avian lymphoid leucosis (virus), pathogens detected by a chicken inoculation test, or a chicken embryo inoculation test, lymphocytic choriomeningitis virus, cytopathic and haemadsorbing agents, and pathogens detected by enzyme-linked immunosorbent assay, polymerase chain reaction, or the fluorescent antibody technique.

2.2. Batch/serial safety test

Batch/serial safety tests are required by some regulatory authorities for the release of each batch/serial and typical tests are described in CFR Title 9 part 113, 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 marketing authorisation 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. The European Pharmacopoeia no longer requires a batch safety test in target animal species for the release of vaccine batches/serials.

2.3. 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 may 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/titre 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/titre will be at least equal to that of the batch/serial 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*.

3. Other tests

3.1. Tests on the finished product

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/lyophilised 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/lyophilised products, and a general physical examination of the final vaccine. A loss of potency may result when residual inactivating agent in a killed liquid product used as a diluent for a desiccated/ lyophilised live fraction reduces the viability of the live organisms because of virucidal or bactericidal activity. Each batch/serial of liquid killed vaccine that is to be used as a diluent for live vaccines must, therefore, be tested for virucidal or bactericidal activity prior to release.

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*.

3.2. Tests on other products

3.2.1. Purity

Purity is determined by testing for a variety of contaminants. Tests to detect contaminants are performed on samples of master seeds, primary cells, master cell stocks (MCS), ingredients of animal origin if not subjected to sterilisation (e.g. fetal bovine serum, bovine albumin, or trypsin).

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 minimise the risk of impurity ~~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.

3.2.2. Tests for the detection of TSE agents

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 in accordance with the *Terrestrial Code*. Methods of production should also document the measures taken to prevent cross contamination of low risk materials by higher risk materials during processing.

MARKET MONITORING

1. Performance monitoring

Marketing authorisation holders or 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 and by the marketing authorisation holder/manufacturer itself. Consumer complaints may serve as one source of information; however, such information needs to be investigated to determine whether 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.

Exporting countries and importing countries should ensure that marketing authorisation holders or manufacturers establish a reliable system to monitor adverse reaction notification (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 on-going and an integral part of all regulatory programmes for veterinary vaccines, especially live vaccines. The marketing authorisation holder or manufacturer plays a big part in the conduct of this continuous overall vaccinovigilance evaluation. When it is determined that a product has a quality defect, immediate action should be taken to notify animal health authorities, to remove the product from the market and, if possible, to inform the end users.

2. 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 marketing authorisation 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 and the public interest. 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~~ legal authorisation 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.

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 related to the manufacturing process. These inspections should be carried out on a regular basis and should allow the assessment of the manufacturing sites with regards to GMP standards.

These inspections may include such items as: personnel qualifications; record keeping; general sanitation and laboratory standards; 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.

Details regarding the inspection of production facilities and requirements for inspectorates are described in chapter 1.1.9.

FURTHER READING

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

CODE OF FEDERAL REGULATIONS (OF THE UNITED STATES OF AMERICA) (CFR) (2000). Title 9, Parts 1–199. US Government Printing Office, Washington DC, USA. http://www.gpo.gov/fdsys/pkg/CFR-2006-title9-vol1/pdf/CFR-2006-title9-vol1-chapI.pdf or ELECTRONIC CODE OF FEDERAL REGULATIONS, accessed at http://www.ecfr.gov/cgi-bin/text-idx?SID=a96ece744f88b16cc39202d9cbc5bdae&tpl=/ecfrbrowse/Title09/9tab_02.tpl

EUROPEAN PHARMACOPOEIA 7.0. (2012). European Directorate for the Quality of Medicines and Health Care (EDQM), Council of Europe, Strasbourg, France.

ESPESETH D.A. (1993). Licensing Veterinary Biologics in the United States. The First Steps Towards an International Harmonization of Veterinary Biologicals; and Free circulation of vaccines within the EEC. *Dev. Biol. Stand.,* **79**, 17–25.

ESPESETH D.A. & GOODMAN J.B. (1993). Chapter 13. *In:* Licensing and Regulation in the USA. Vaccines for Veterinary Application. Butterworth Heinemann, London, UK, 321–342.

EUROPEAN COMMISSION (2006). The Rules Governing Medicinal Products in the European Union. Eudralex. Volumes 1–9. European Commission Enterprise and Industry DG; Directorate F – Consumer goods. Latest versions only available at http://pharmacos.eudra.org/F2/eudralex/index.htm.

GAY C.G. & ROTH H.J. (1994). Confirming the safety characteristics of recombinant vectors used in veterinary medicine: a regulatory perspective. Recombinant vectors in vaccine development. *Dev. Biol. Stand.*, **82**, 93–105.

ROTH H.J. & GAY C.G. (1996). Specific safety requirements for products derived from biotechnology. *In:* Veterinary Vaccinology, Pastoret P.-P., Blancou J., Vannier P. & Verschueren C., eds. Elseviers Science Publishers B.V. Amsterdam, The Netherlands.

PASTORET P.P., BLANCOU J., VANNIER P. & VERSCHUEREN C., EDS (1997). Veterinary Vaccinology. Elsevier Science, Amsterdam, The Netherlands.

PIC/S GUIDE AVAILABLE AT THE FOLLOWING ADDRESS: WWW.PICSCHEME.ORG

USDA-APHIS[2]-VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1999). Categories of Inspection for Licensed Veterinary Biologics Establishments. Veterinary Services Memorandum No. 800.91. Center for Veterinary Biologics, 510 S. 17th Street, Suite 104, Ames, Iowa 50010, USA.

USDA-APHIS-VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1999). Veterinary Biological Product Samples. Veterinary Services Memorandum No. 800.59. Center for Veterinary Biologics, 510 S. 17th Street, Suite 104, Ames, Iowa 50010, USA.

USDA-APHIS- VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1995). Guidelines for Submission of Materials in Support of Licensure. Veterinary Biologics Memorandum No. 800.84. Center for Veterinary Biologics, 510 S. 17th Street, Suite 104, Ames, Iowa 50010, USA.

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.

USDA-APHIS-VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1995). Veterinary Biologics General Licensing Considerations No. 800.201, Back Passage Studies. Center for Veterinary Biologics, 510 S. 17th Street, Suite 104, Ames, Iowa 50010, USA.

2 United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS). USDA-APHIS-Center for Veterinary Biologics Home Page: http://www.aphis.usda.gov/vs/cvb/index.html

547 USDA-APHIS-VETERINARY SERVICES (1964–1994). Standard Assay Methods, Series 100–900. National Veterinary
548 Services Laboratories, Ames, Iowa 50010, USA.

549 USDA-APHIS- VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1984). Basic License Requirements for
550 Applicants. Veterinary Biologics Memorandum No. 800.50. Center for Veterinary Biologics, 510 S. 17th Street,
551 Suite 104, Ames, Iowa 50010, USA

552 USDA-APHIS-VETERINARY SERVICES-CENTER FOR VETERINARY BIOLOGICS (1988). Guidelines for the Preparation and
553 Review of Labeling Materials. Veterinary Services Memorandum No. 800.54. Center for Veterinary Biologics, 510
554 S. 17th Street, Suite 104, Ames, Iowa 50010, USA.

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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~~ MARKETING AUTHORISATION 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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