

MINIMUM REQUIREMENTS FOR THE PRODUCTION AND QUALITY CONTROL OF VACCINES

SUMMARY

This chapter provides requirements for the manufacture and quality control of veterinary vaccines in accordance with Chapter 1.1.8 Principles of veterinary vaccine production. Manufacturers should use the recommendations as a basis for the elaboration of specific rules adapted to their individual needs.

Production operations should follow clearly defined procedures that comply with the principles elaborated in this chapter, in order to obtain products of the requisite quality in accordance with the marketing authorisations. The manufacture of immunological veterinary medicinal products has special characteristics that should be considered 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. The products should be protected against organic or inorganic contamination and cross-contamination. The environment must also be protected, especially when using pathogenic or exotic biological agents, and personnel must be protected from biological agents pathogenic to humans. The role of the quality assurance system is therefore of paramount importance.

Quality control is concerned with sampling, specifications and testing as well as the organisation, documentation and release procedures to ensure that the necessary and relevant tests are carried out, and that materials 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. That is, production or management personnel should not coerce or force quality assurance personnel to depart from approved specifications or procedures.

1. General requirements for vaccine production

1.1. Principle

Production operations must follow clearly defined approved current procedures; they must comply with the following principles in order to obtain products of the requisite quality and be in accordance with the relevant manufacturing and marketing authorisations.

The manufacture of immunological veterinary medicinal products has special characteristics that 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 campaign basis is common, manufacturing a series of batches of the same product in sequence in a given period of time followed by an appropriate cleaning procedure. Moreover, because of the very nature of this manufacture (cultivation steps, lack of terminal sterilisation), the products must be particularly well protected against organic or inorganic contamination and cross-contamination. The environment also must 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 humans.

These factors, together with the inherent variability of immunological products and the destructive nature of final product quality control tests so that they can provide only an estimate of quality for the entire batch mean that the role of the quality assurance system is of the utmost importance.

1.2. General

- i) Production should be performed and supervised by competent people. Workers must understand the theory behind and practice of their work to the extent that they can predict and prevent problems within the scope of their responsibility.
- ii) All handling of materials and products, such as receipt and quarantine, sampling, storage, labelling, dispensing, processing, packaging and distribution should be done in accordance with approved written procedures or instructions and adequately documented.
- iii) All incoming materials should be evaluated to assess the quality impact on manufacturing. Appropriate action should be taken when materials are determined to be compromised. Materials should be labelled with the prescribed specifications.
- iv) Incoming materials and finished products should be physically or administratively quarantined immediately after receipt or processing, until they have been released for use or distribution.
- v) Intermediate and bulk products purchased as such should be handled on receipt as though they were starting materials.
- vi) All materials and products should be stored under the appropriate conditions as established by the manufacturer and in an orderly fashion to permit batch segregation and stock rotation according to life expectancy.
- vii) Checks on yields, and reconciliation of quantities, should be carried out as necessary to ensure that there are no discrepancies outside acceptable limits.
- viii) Operations on different products should not be carried out simultaneously or consecutively in the same room unless there is negligible risk of mix-up or cross-contamination.
- ix) At every stage of processing, products and materials should be protected from microbial and other contamination. A method of measuring bioburden within the production facility should be established.
- x) When working with dry materials and products, special precautions should be taken to prevent the generation and dissemination of dust particulates. This applies particularly to the handling of highly active or sensitising materials.
- xi) At all times during processing, all materials, bulk containers, critical items of equipment and where appropriate rooms used should be labelled or otherwise identified with an indication of the product or material being processed, its strength or concentration (where applicable) and batch number. Where applicable, this indication should also mention the stage of production.
- xii) Labels applied to containers, equipment or premises should be clear, unambiguous and in the company's approved format. It is often helpful in addition to the wording on the labels to use colours to indicate status (for example, quarantined, accepted, rejected, clean, etc.).
- xiii) Checks should be carried out to ensure that pipelines and other pieces of equipment used for the transportation of products from one area to another are connected in a correct manner.
- xiv) Any deviation from instructions or procedures should be avoided as far as possible. If a deviation occurs, it should be approved in writing by a competent authorised person, with the involvement of the quality control department as appropriate to evaluate the effect on product quality and the shelf life of the product. Deviations from documents approved by regulatory bodies should be reported to them for written approval prior to release of product.
- xv) Access to production premises should be restricted to authorised personnel.

- xvi) Normally, the production of other products should be avoided in areas destined for the production of vaccines, and should not use the same equipment.

1.3. Prevention of cross-contamination in production

- i) Contamination of a starting material or of a product by another material or product should be avoided. This risk of accidental cross-contamination arises from the uncontrolled release of dust, gases, vapours, sprays or organisms from materials and products in process, from residues on equipment, residues from excipients or packaging and from operators' clothing, skin and respiratory tract. The significance of this risk varies with the type of contaminant and of product being contaminated. Amongst the most hazardous contaminants are biological preparations containing living organisms.
- ii) Cross-contamination should be avoided by appropriate technical or organisational measures, for example:
 - a) production in segregated areas or by campaign (separation in time) followed by appropriate cleaning;
 - b) providing appropriate air-locks and air extraction;
 - c) minimising the risk of contamination caused by recirculation or re-entry of untreated or insufficiently treated air; routine testing of air;
 - d) keeping protective clothing inside areas where products with special risk of cross-contamination are processed;
 - e) using cleaning and decontamination procedures of known effectiveness, as ineffective cleaning of equipment is a common source of cross-contamination;
 - f) using "closed systems" of production;
 - g) testing for residues and contamination, and use of cleaning status labels on equipment.
- iii) Measures to prevent cross-contamination and their effectiveness should be checked periodically according to set procedures.

1.4. Starting materials

- i) The suitability of starting materials should be clearly defined in written specifications. These should include details of the supplier, catalogue or part number, the method of manufacture, the geographical origin and the animal species from which the materials are derived. The controls to be applied to starting materials must be included. Microbiological controls are particularly important.
- ii) The results of tests on starting materials must comply with the specifications. Where the tests take a long time (e.g. eggs from SPF flocks) it may be necessary to process starting materials before the results of analytical controls are available. In such cases, the release of a finished product is conditional upon satisfactory results of the tests on starting materials. While vendors' certificates of analysis are useful and may be an acceptance requirement, they should not replace testing by the manufacturer when those test results are critical to acceptance.
- iii) Special attention should be paid to knowledge of the supplier's quality assurance system in assessing the suitability of a source and the extent of quality control testing required.
- iv) Heat is the preferred method for sterilising starting materials and some equipment. If necessary, other validated methods, such as irradiation, may be used. Steam heat should use live steam at no less than 120°C for not less than 30 minutes. Dry heat should be at no less than 160°C for not less than 1 hour.

1.5. Media

- i) The ability of media to support the desired growth and effectiveness should be properly validated in advance.
- ii) Media should preferably be sterilised *in situ* or in line. Steam heat under pressure is the preferred method. Gases, media, acids, alkalis, de-foaming agents and other materials introduced into bioreactors should themselves be sterile.

1.6. Seed lot and cell bank system

Where appropriate, the standards for seed lot and cell bank systems in relation to specific disease chapters in the *Terrestrial Manual* should be consulted.

- i) In order to prevent the unwanted drift of properties that ensue from repeated subcultures or multiple generations, the production of immunological veterinary medicinal products obtained by microbial, cell or tissue culture, or propagation in embryos and animals, should be based on a system of limited and controlled passages of seed lots or cell banks with a specified maximum.
- ii) The number of generations (doublings, passages) between the seed lot or cell bank and the finished product should be consistent with the relevant dossier regulatory approval.
- iii) Seed lots and cell banks should be adequately characterised and tested for contaminants (freedom from extraneous bacteria, fungi, mycoplasma, and viruses) and to ensure identity, purity, safety and efficacy when required by regulators. Cell banks should also be tested for karyology at baseline and maximum passage. Regulatory bodies may require confirmatory testing. Acceptance criteria for new seed lots should be established. Seed lots and cell banks should be established, stored and used in such a way as to minimise the risks of contamination, or any alteration. During the establishment of the seed lot and cell bank, no other living or infectious material (e.g. virus or cell lines) should be handled simultaneously in the same area or by the same person.
- iv) Establishment of the seed lot and cell bank should be performed in a suitable environment to protect the seed lot and the cell bank and, if applicable, the personnel handling it and the external environment. The master seed or the cell bank should consist of a single uniform batch or serial that has been mixed and filled into containers as one batch or serial.
- v) Only authorised personnel should be allowed to handle the material and this handling should be done under the supervision of a competent person. Different seed lots or cell banks should be stored at the temperature that assures negligible degradation while taking care to avoid confusion or cross-contamination errors. It is desirable to split the seed lots and cell banks and to store the parts at different locations so as to minimise the risk of total loss. Storage equipment should be monitored for proper function and connected to an alarm system for immediate notification of malfunction.

1.6.1. Master seeds and working seeds

- i) A master seed (reference culture, parental strain) 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. Records of the source of the master seed should be maintained. For each seed, the highest and lowest passage levels that may be used for production should be established and specified in the approved production documents for the relevant regulatory procedure.
- ii) Working seeds and production seeds may be prepared from the master seed by subculturing. Using a master seed and limiting the number of passages of seed microorganism in this manner assists in maintaining uniformity and consistency in production.
- iii) The origin, form and storage conditions of seed material should be described (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). Storage containers should be adequately sealed and clearly labelled. Storage conditions should be properly monitored. An inventory should be kept and each container accounted for. Tamper evident tape may be needed for boxes and containers.
- iv) 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.

1.6.2. Master cell stocks

- i) 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 approved

documents. 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 tumorigenicity 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.

1.6.3. Primary cells

- i) 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.
- ii) In the case of products for use in poultry, these cells are usually obtained from SPF embryonating chicken eggs that have originated in an unvaccinated flock subjected to intensive microbiological monitoring.
- iii) 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 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.

1.6.4. Embryonated eggs

- i) Embryonated eggs are also commonly used in the production of biologicals. They should be derived from SPF chicken flocks that have been intensively monitored for infectious agents and have not been vaccinated; or, where justified (e.g. for production of some inactivated vaccines) and in line with the marketing authorisation, from healthy chicken flocks. 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. Regulatory bodies may have requirements for sources of eggs, and for release testing of products grown on them.

1.7. Operating principles

- i) The formation of droplets and the production of foam should be avoided or minimised during manufacturing processes. Centrifugation and blending procedures that can lead to droplet formation should be carried out in appropriate contained or clean or contained areas to prevent transfer of live organisms.
- ii) Accidental spillages, especially of live organisms, must be dealt with quickly and safely. Validated decontamination procedures should be available for each organism. Where different strains of single bacterial species or very similar viruses are involved, the process needs to be validated against only one of them, unless there is reason to believe that they may vary significantly in their resistance to the agent(s) involved.
- iii) Operations involving the transfer of materials such as sterile media, cultures or products, should be carried out in pre-sterilised closed systems wherever possible. Where this is not possible, transfer operations must be protected by laminar airflow work stations.
- iv) Addition of media or cultures to biogenerators (fermenters) and other vessels should be carried out under carefully controlled conditions to ensure that contamination is not introduced. Care must be taken to ensure that vessels are correctly connected when addition of cultures takes place.
- v) Where necessary, for instance when two or more fermenters are within a single area, sampling and addition ports, and connectors (after connection, before the flow of product, and again before disconnection) should be sterilised with steam.
- vi) Documentation, equipment, glassware, the external surfaces of product containers and other such materials must be disinfected before transfer from a contained area using a validated method. Only the absolute minimum required to allow operations to GMP standards should enter and leave the area. If obviously contaminated, such as by spills or aerosols, or if the organism involved is an exotic, the paperwork must be adequately disinfected through an equipment pass, or the information transferred out by such means as photocopy or fax.

- vii) Liquid or solid wastes such as the debris after harvesting eggs, disposable culture bottles, unwanted cultures or biological agents, are best sterilised or disinfected before transfer from a contained area. However, alternatives such as sealed containers or piping may be appropriate in some cases.
- viii) Articles and materials, including documentation, entering a production room should be carefully controlled to ensure that only items concerned with production are introduced. There should be a system that ensures that articles and materials entering a room are reconciled with those leaving so that their accumulation within the room does not occur.
- ix) Heat-stable articles and materials entering a clean or contained area should do so through a double-ended autoclave or oven. Heat-labile articles and materials should enter through an airlock with interlocked doors where they are disinfected. Sterilisation of articles and materials elsewhere is acceptable provided that they are double wrapped and enter through an airlock with the appropriate precautions.
- x) Precautions must be taken to avoid contamination or confusion during incubation. There should be a cleaning and disinfection procedure for incubators. Containers in incubators should be carefully and clearly labelled.
- xi) With the exception of blending and subsequent filling operations (or when totally enclosed systems are used) only one live biological agent may be handled within a production room at any given time. Production rooms must be effectively disinfected between the handling of different live biological agents.
- xii) Products should be inactivated by the addition of inactivant accompanied by sufficient agitation, with specified time and conditions. The mixture should then be transferred to a second sterile vessel, unless the container is of such a size and shape as to be easily inverted and shaken so as to wet all internal surfaces with the final culture or inactivant mixture.
- xiii) Vessels containing inactivated products should not be opened or sampled in areas containing live biological agents. All subsequent processing of inactivated products should take place in clean areas (as defined in the paragraph related to aseptic preparation) or in enclosed equipment dedicated to inactivated products.
- xiv) Methods for sterilisation, disinfection, virus removal and inactivation should be validated.
- xv) Filling should be carried out as soon as possible following production. Containers of bulk product prior to filling should be sealed, appropriately labelled and stored under specified conditions of temperature.
- xvi) There should be a system to assure the integrity and closure of containers after filling.
- xvii) The capping of vials containing live biological agents must be performed in such a way that ensures that contamination of other products or escape of the live agents into other areas or the external environment does not occur.
- xviii) There may be a delay between the filling of final containers and their labelling and packaging. Procedures should be specified for the storage of unlabelled containers in order to maintain process control and to ensure satisfactory storage conditions. Special attention should be paid to the storage of heat-labile or photosensitive products. Storage temperatures should be specified and monitored.
- xix) For each stage of production, the yield of product should be reconciled with that expected from that process. Any unexpected discrepancies should be investigated.

2. Rules governing quality control

2.1. Principle

Quality control is concerned with sampling, specifications and testing as well as the organisation, documentation and release procedures that ensure that the necessary and relevant tests are carried out, and that materials 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.

2.2. General rules for veterinary medicinal products including vaccines

- i) Each holder of a relevant regulatory approval should have a quality control department. This department should be independent from other departments, and under the authority of a person with appropriate qualifications, who has adequate laboratory support. Adequate resources must be available to ensure that all the quality control requirements are effectively and reliably carried out.
- ii) The head of the quality control department generally has the following responsibilities:
 - a) to approve or reject, as he/she sees fit, starting materials, packaging materials, and intermediate, bulk and finished products;
 - b) to evaluate batch records;
 - c) to ensure that all necessary testing is carried out;
 - d) to approve specifications, sampling instructions, test methods and other quality control procedures;
 - e) to approve and monitor any contract analysts;
 - f) to check the maintenance of his/her department, premises and equipment;
 - g) to ensure that the appropriate validations are done;
 - h) to ensure that the required initial and continuing training of department personnel is carried out and adapted according to need.
- iii) The quality control department may have other duties, such as to establish, validate and implement all quality control procedures, keep the reference samples of materials and products, provide training and SOPs or Directives to departments to ensure the correct labelling of containers of materials and products, ensure the monitoring of the stability of the products, and participate in the investigation of complaints related to the quality of the product. All these operations should be carried out in accordance with written procedures and recorded.
- iv) Finished product assessment should include all relevant factors, including production conditions, results of in-process testing, a review of manufacturing (including packaging) documentation, compliance with finished product specifications and examination of the finished product.
- v) In-process controls ensure the quality of product. Those controls should be performed at an appropriate stage of production.
- vi) There may be a requirement for the continuous monitoring of data during a production process, for example monitoring of physical parameters during fermentation.
- vii) Continuous culture of biological products is a common practice and special consideration needs to be given to the quality control requirements arising from this type of production method.

2.3. Good practice for quality control in laboratories

- i) Control laboratory premises and equipment should meet the general and specific requirements for quality control areas given in this chapter.
- ii) The personnel, premises, and equipment should be appropriate to the tasks imposed by the nature and the scale of the manufacturing operations. The use of outside laboratories, in conformity with the principles detailed in Chapter 3.7.1 *Minimum requirements for the organisation and management of a vaccine manufacturing facility*, Section 4. *Rules governing outsourced activities*, can be accepted for particular documented reasons.

2.3.1. Documentation

- i) Laboratory documentation should follow the principles given in chapter 3.7.1, Section 3. *Rules governing documentation*. The following details should be available to the quality control department:

- a) specifications;
 - b) sampling procedures;
 - c) testing procedures and records (including analytical worksheets or laboratory notebooks);
 - d) analytical reports or certificates;
 - e) data from environmental monitoring, where required;
 - f) validation records of test methods, where applicable;
 - g) procedures for and records of the calibration of instruments and maintenance of equipment.
- ii) Any quality control documentation relating to a batch record should be retained for one year after the expiry date of the batch or at least 5 years after the certification. Records retention requirements may be specified by the relevant regulatory body or national laws.
 - iii) For some kinds of data (e.g. analytical tests results, yields, and environmental controls) it is recommended that records are kept in a manner permitting trend evaluation.
 - iv) In addition to the information that is part of the batch record, other original data such as laboratory notebooks or records should be retained and readily available

2.3.2. Sampling

- i) Sampling should be done in accordance with approved written procedures that describe:
 - a) the method of sampling;
 - b) the equipment to be used;
 - c) the amount of the sample to be taken;
 - d) instructions for any required sub-division of the sample;
 - e) the type and condition of the sample container to be used;
 - f) the identification of containers sampled;
 - g) any special precautions to be observed, especially with regard to the sampling of sterile or noxious materials;
 - h) the storage conditions;
 - i) Instructions for the cleaning and storage of sampling equipment.
- ii) Quality control personnel should have access to production areas for sampling and investigation.
- iii) Samples are retained; firstly to provide a sample for analytical testing and secondly to provide a specimen of the fully finished product. Samples may therefore fall into two categories:
 - a) *Reference sample*: a sample of a batch of starting material, packaging material or finished product that is stored for the purpose of being analysed should the need arise during the shelf life of the batch concerned.
 - b) *Retention sample*: a sample of a fully packaged unit from a batch of finished product.

These are stored for identification and retest purposes during or beyond the shelf life of the product. The number of retention samples may be specified by the relevant regulatory authority, otherwise they should be stored at least in duplicate.
- iv) Samples should be selected from each batch or serial of product. The selector should pick representative final containers from each batch or 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 12 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.
- v) It may be necessary to retain samples of intermediate products in sufficient amount and under appropriate storage conditions to allow repetition or confirmation of a batch control.

- vi) Samples should be representative of the batch of materials or products from which they are taken. Other samples may also be taken to monitor the most stressed part of a process (e.g. beginning or end of a process).
- vii) Sample containers should bear a label indicating the contents, the batch number, the date of sampling and the containers from which samples have been drawn.

2.3.3. Testing

- i) Analytical methods should be validated. All testing operations described in the relevant regulatory approval documents should be carried out according to the approved methods.
- ii) The results obtained and associated calculations should be checked and recorded as satisfactory or not. If not, action should be taken according to the manufacturer's procedures.
- iii) Records should include at least the following data:
 - a) name of the material or product and, where applicable, dosage form;
 - b) batch number and, where appropriate, the manufacturer or supplier;
 - c) references to the relevant specifications and testing procedures;
 - d) test results, including observations and calculations, and reference to any certificates of analysis;
 - e) dates of testing;
 - f) initials of the persons who performed the testing;
 - g) initials of the persons who verified the testing and the calculations, where appropriate;
 - h) a clear statement of release or rejection (or other status decision) and the dated signature of the designated responsible person.
- iv) All the in-process controls and procedures should be performed according to methods approved by quality control and the results recorded.

2.4. Batch tests for immunological products

- i) It may be necessary to retain samples of intermediate products in sufficient amount and under appropriate storage conditions to allow repetition or confirmation of a batch control.
- ii) There may be a requirement for the continuous monitoring of data during a production process, for example monitoring of physical parameters during fermentation.
- iii) Continuous culture of biological products is a common practice and special consideration needs to be given to the quality control requirements arising from this type of production method.

2.4.1. Batch or serial release for distribution

- i) Prior to release, the manufacturer must test each batch or serial for purity, (safety if required), 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 programs that include official control authority re-testing (check testing) of final products, samples of each batch or 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 or serial should not be released. In such cases, subsequent batches or serials of the product should be given priority for check testing by competent authorities.

2.4.1.1. Batch or serial purity test

- i) Purity is determined by testing for a variety of contaminants. Tests to detect contaminants are performed on: master seeds, primary cells, MCSs, ingredients of animal origin if not subjected to sterilisation (e.g. fetal bovine serum, bovine albumin, or trypsin), and each batch or serial of final product prior to release.
- ii) Purity test procedures detailed in Chapter 1.1.9 *Tests of biological materials for sterility and freedom from contamination*.

- iii) Procedures used to provide evidence that fetal or calf serum and other ingredients of bovine origin are free of pestiviruses are of great importance and should be well documented.
- iv) Tests to be used to provide evidence of purity vary with the nature of the product, and should be prescribed in the Outline of Production or other documentation of the manufacturing process.
- v) 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:

- a) first, verification that the animal source of all ingredients of animal origin in production facilities are from countries recognised as having the lowest possible risk of bovine spongiform encephalopathy;
- b) second, that the tissues or other substances used are themselves recognised as being of low or nil risk of containing TSE agents;
- c) 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.

2.4.1.2. Batch or serial safety test

- i) Safety tests are not required by many regulatory authorities for the release of each batch or serial where the seed-lot system is used. Other regulatory authorities may allow waiving of target animal batch safety tests in line with VICH GL50 and 55.
- ii) Where required, standard procedures are used 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 or serials are considered satisfactory if local and systemic reactions to vaccination with the batch or serial to be released are in line with those described in the relevant regulatory approval dossier and product literature.

2.4.1.3. Batch or serial potency test

- i) Batch or serial potency tests, required for each batch or serial prior to release, are designed to correlate with the host animal vaccination–challenge efficacy studies.
- ii) 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.
- iii) The potency of live vaccines is generally measured by means of bacterial counts or virus titration.
- iv) 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.
- v) 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.
- vi) 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.
- vii) 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.

- viii) 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*.

2.4.2. Other tests

- i) 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:

- a) The level of moisture contained in desiccated products,
- b) The level of residual inactivant in killed products,
- c) The complete inactivation of killed products, pH,
- d) The level of preservatives and permitted antibiotics,
- e) The physical stability of adjuvants,
- f) The retention of vacuum in desiccated products,
- g) A general physical examination of the final vaccine.

Tests for these purposes may also be found in this *Terrestrial Manual*.

- ii) Samples taken for sterility testing should be representative of the whole of the batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, e.g. for products that have been filled aseptically, samples should include containers filled at the beginning and end of the batch and after any significant intervention,
- iii) The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. The test should be validated for the product(s) concerned.

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NB: FIRST ADOPTED IN 2016. MOST RECENT UPDATES ADOPTED IN 2018.