Medicines Control Authority of Zimbabwe

GUIDELINES FOR SUBMISSION OF DOCUMENTATION FOR REGISTRATION OF VETERINARY VACCINES IN ZIMBABWE

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Applications already submitted are being evaluated using this guideline.

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Table of Contents

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PROCESS AND ADOPTION OF THE GUIDELINE

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Introduction

These guidelines have been drafted for applicants interested in having their veterinary vaccines evaluated for registration in Zimbabwe. Applicants should submit a product dossier reflecting the data and information requested in this guideline. The current version of any guideline or pharmacopoeia referred to in this guideline shall be applicable in all instances. Any deviations must be highlighted, justified and require approval by the MCAZ.

The present guidelines have been prepared taking into consideration the need for worldwide harmonization, which will assist the medicine manufacturers in the preparation of a well-structured dossier to be submitted for the registration of veterinary vaccines in order to facilitate their screening and subsequent review.

This guideline provides details about the type of Quality information concerning the Manufacture and Control of veterinary vaccines that the applicant should present in the registration dossier[1]. It also describes the data required to support the Safety and Efficacy of the product. In addition to these sections on Quality, Safety and Efficacy the applicant must include the documents described under Part 1 of the Dossier Structure[1] document.

In addition to the sections of the dossier covered by this guideline, the applicant is required to complete the Application Form (MC8 available on: (http://www.mcaz.co.zw/index.php/downloads/category/12-feedback-forms) and include it in Part 1 of the dossier. Draft Packaging and the SPC should also be included in the Part 1.

PART 1: PRODUCT INFORMATION

1. A Administrative Information

This section should include the completed and signed MC8 Application Form, including the name and brief description of the product, name and address of the applicant, the name and address of the manufacturer and the list of countries where the veterinary vaccine is already registered.

Cover letter
The covering letter submitted with the application for registration should include a clear statement by the responsible person submitting the dossier, indicating the contact details (telephone number, e-mail, and fax) of the person to whom all correspondences should be addressed.

Declaration by the applicant
A declaration should be made by the applicant or a responsible person nominated by the applicant and who must have the requisite skills and necessary qualifications. It is stressed that only a person who can attest to the accuracy of the contents in the application should sign on behalf of the applicant. False / misleading declarations will lead to prosecution.

Failure to make the declaration will lead to the rejection of the application.

Screening checklist
A screening checklist in Annex II should be completed by the applicant. The Authority will assess the application for completeness upon submission before it is accepted for evaluation by MCAZ. Incomplete applications will be rejected and the applicant requested to submit a complete application. The Authority may charge a re-submission fee for products that fail screening.

Proof of payment of appropriate fees
A copy of the invoice or proof of payment of registration fees should be attached to the application for registration. Applicants should consult the current fee schedule for the correct and appropriate fee. Note that registration fees can be split into three main tiers, depending on the site of manufacture of the vaccine. Fees for products wholly manufactured outside Zimbabwe attract a different fee from products wholly or partly (e.g. re-labelled and re-packaged) manufactured in Zimbabwe. All fees are payable according to the gazetted fee schedule
Unless the full application fee is received, the application will not be accepted.

Applicants can remit payment of fees in cash, telegraphic transfers and direct deposit into the Authority account. Please note that direct transfers usually attract a commission charged by

the banks leading to a shortfall in fees. Provision should, therefore, be made for all bank charges to be borne by the applicant's bank account.
Applicants are further advised to specify very clearly in their instructions to the bank that such direct deposits are for application for registration of a medicine to avoid unnecessary delays.

Note that the application fee covers the cost of evaluating the initial submission and a single laboratory analysis of the product sample. Samples that require repeat analysis after failure of the first analysis, or as a result of modification or revalidation of the analytical method, attract an additional re-analysis fee. Any amendments to the original submission will attract amendment fees according to the gazetted fee schedule. The application fee excludes the GMP inspection fees, for which a separate charge is applicable.

Fees once received are not refundable, including those for rejected applications or voluntary withdrawals by the applicant.

Manufacturing and Marketing authorization(s) / international registration status

List the countries in which:
• the Veterinary vaccine has been granted a marketing authorisation;
• the vaccine (or one or more of the set of vaccines) has been withdrawn from the market; and
• an application for the marketing of the vaccine (or one or more of the set of vaccines) has been rejected, deferred or withdrawn

The details of registration in the country of origin are required. Reasons for non-registration should be stated if the medicine is not registered in the country of origin.
Registration status in the country of manufacture should be indicated including any withdrawal, cancellations, suspension / revocations. The reasons for these should also be indicated.

1. B Summary of Product Characteristics (SPC), Primary container label, secondary container text (carton) and Packaging leaflet

1. B.1 Summary of Product Characteristics

Copies of all package inserts and any information intended for distribution with the product should be submitted. These should be written in English, be legible and comprehensible.

The Authority will determine the appropriate category for distribution of a medicine as set out in the sixth schedule of the Medicines and Allied Substances Control Regulations (Statutory Instrument 150 of 1991).

The categories are:
a) Narcotic Medicines or Dangerous Drug (abbreviated as "N") – products containing ingredients stated as such in the legislation and which may be subject to control by the International Narcotics Board.
b) Prescription Preparations (P.P. Vet) – medicines belonging to this category are

available on prescription only.
c) Household Remedies (H.R. Vet) - Medicines in this category are available to end-users through supermarkets.
d) Veterinary Medicines (General Dealer) (V.M.G.D.) – veterinary medicines in this category are available in shops that hold Veterinary Medicines General Dealer permits from MCAZ.

Name of product
Trade name of product should be provided. The proposed proprietary name of the product should not infringe on the INN stem. It should not imply superiority over other products. It should not be the same or similar to the name of another medicine to avoid confusion and dispensing errors.

Name of Active Substance(s)/API
[use International Non-proprietary Name (INN), if any], dosage form and strength of the product The English INN of all the active ingredients, dosage form and the strength in the formulation should be given.

Target Species
Applicant should indicate target species for which the medicine is intended.

Visual description of the Vaccine
E.g.,White freeze dried pellet containing live virus vaccine

Description of pack sizes and pack type
Visual description of the pack sizes and pack types.

Primary packing materials
Primary packaging components are those that are in direct contact with the vaccine.

The material of construction of the immediate container should be stated (’Type I glass vials’, ampoules, aerosol canisters).
The container of any diluent provided with the medicinal product should also be described.

Secondary packing
For non-functional secondary packaging components (e.g. those that do not provide additional protection), only a brief description should be provided. For functional secondary packaging components, additional information should be provided.

Sample
Provide a sample of the vaccine(s) to enable visual inspection of the vaccine (s). The quantity of samples submitted to the Authority for laboratory analysis should be, in general, at 2 samples packaged in the primary packaging material

Details of applicant
The name and addresses (physical and postal) for applicant, including e-mail, fax, telephone numbers and cellular phone numbers.

Details of Manufacturer(s) of the Vaccine
The name and addresses (physical and postal) for all the manufactures involved in the manufacture of the product are to be indicated. The steps of manufacturing process performed should also be indicated for each site. The details of any contract company used at development of the formulation, preclinical or clinical study trials should be indicated.

Route of administration
The route of administration e.g. oral, IM injection, rectal, should be indicated

Container, closure and administration devices
The type(s) of the container/closure and administration devices should be stated.

Shelf life
The proposed shelf life of each dosage form in each of the different package type(s) and sizes should be stated.
• the shelf life after first opening of container should be indicated.
• the shelf life after reconstitution with a diluent should be indicated

Storage conditions
The conditions under which the finished product should be stored, e.g. store in a cool dark place between 2-8°C, should be stated.

Proposed indications
The uses for which the vaccine is being registered should be indicated.

Withdrawal periods
The applicants should state the withdrawal periods supported by experimental data. The withdrawal periods should be comparable with that of the reference product (for milk refer as the Withholding Time) this is the length of time that must elapse after treating an animal with a vaccine before the animal or its products can be marketed. Withdrawal Period/Withholding Time varies for different products, reflecting the amount of time needed for an animal to metabolize that drug and for the drug's concentration level in the animal tissue or product to decrease to a safe, acceptable level.

1. B.2 Primary container label, secondary container text (carton)

Labelling
The information and instructions on the labels of the test product should be the same as the reference product. Fewer or reduced claims compared to the reference product may be acceptable as long as the partial claim does not raise any potential safety and efficacy concerns. The applicant is requested to submit the most recent English versions of all the reference product labels (inner and outer labels and package inserts) along with the proposed English draft labels [hard copy and electronic format (if available)].

The label of the primary container for each vaccine products shall meet the WHO Good Manufacturing Practices: Main Principles for Pharmaceutical Products, (WHO Technical Report Series, No. 908, 2003)

The W210 GMP standard and include:

- The international non-proprietary name (INN) or generic name prominently displayed and above the brand name, where a brand name has been given. Brand names should not be bolder or larger than the generic name;
- Dosage form, e.g. tablet, ampoule, etc.;
- The active ingredient "per unit, dose, tablet or capsule, etc."
- The applicable pharmacopoeia standard;
- The manufacturer's logo and code number and any specific colour coding if required;
- Content per pack;
- Instructions for use;
- Special storage requirements;
- Batch number;
- Date of manufacture and date of expiry (in clear English language, not code);
- Name and address of manufacture;
- Category for distribution; and
- Any additional cautionary statement.

The outer case or carton should also display the above information.

- All cases should prominently indicate the following:
- Manufacturer's line and code numbers;
- The generic name of the product;
- The dosage form (ampoule,vial);
- Date of manufacture and expiry (in clear English language);
- Batch number;
- Quantity per case;
- Special instructions for storage;
- Name and address of manufacture; and
- Any additional cautionary statements.

1. B.3 Package leaflet

Information same as Summary of Product Characteristics (SmPC).

PART 2: QUALITY: MANUFACTURE AND CONTROL

2.A Quantitative and Qualitative Particulars

2.A.1 Table of qualitative and quantitative composition

A tabulated list of all components of the veterinary vaccine and diluents (if applicable) should be given as per table 1 below. The quantities per dose should be stated. A clear description of the active immunogenic substance including the name(s) or designation of the strain of

organism used to produce the active immunogenic substance should be provided. The reason(s) for inclusion of each excipient and a justification for overages should also be stated.

Where applicable; special characteristics of excipients should be indicated. The type of water (e.g purified, demineralised), where relevant, should be indicated.

Table 1: Composition of the Veterinary vaccine

1. Active (immunogenic) ingredients

Name	Quantity per dosage unit	Specification or reference text

2. Inactive ingredients (adjuvant/excipients/preservative)

Name	Quantity per unit dose	Specification or reference text	Reason for inclusion

2.A.2 Containers

Details of the container and closure system, and its compatibility with the immunogenic veterinary product shall be submitted. Detailed information concerning the supplier(s), address(es), and the results of any relevant information on compatibility, toxicity and biological tests shall be provided for containers of novel origin. For sterile product, evidence of container and closure integrity shall be provided for the duration of the proposed shelf life.

Drawings of the containers and closures should be included in the Appendix to Part 2.

2.B Method of Manufacture

2.B.1 Flow chart

A complete visual representation of the manufacturing process flow shall be provided for each active immunogenic substance and the veterinary vaccine. Show the steps in production, including incubation times and temperatures, equipment and materials used the area where the operation is performed and a list of the in-process controls and finished product tests performed at each step. In-process holding steps should be included with time and temperature limits indicated.

2.B.2 Detailed description of manufacture

Provide a description of manufacturing starting with the Working Seed, and including any steps in which the bulk of the active immunogenic substance is further processed (e.g separated from the cells, concentrated). List all the components used in the manufacturing process including media, solvents or solutions etc.

A description shall be provided for:

-Propagation and Harvest
For each antigen production method or combination of methods, a growth curve or tabular representation of growth characteristics for each propagation step shall be provided. Include a table showing yield, purity and viability (if applicable) of the crude harvest.

-Inactivation (if appropriate)
Inactivation kinetics or killing curves, or a tabular representation shall be provided.
Validation of the titration method used to measure residual live organisms, including the sensitivity of the method in a background of inactivating agents, shall be provided. The following information shall be provided:

a) How culture purity is verified before inactivation
b) The method(s) and agent(s) used for inactivation
c) The method(s) undertaken to prevent aggregation and assure homogeneous access of inactivating agent(s) to the culture
d) The stage in production where inactivation or killing is performed
e) The parameters which are monitored

-Detoxification (if appropriate)
For toxoid or toxoid-containing vaccines, the detoxification procedures should be described in detail for the toxin component(s):

a) The method(s) and agent(s) used for detoxification
b) The stage in production where detoxification is performed and the parameters, which are monitored, must be described.

-Purification (if appropriate)
Describe any purification methods used, including specialised equipment such as columns, ultracentrifugation, ultra-filtration, and custom reagents such as monoclonal antibodies. State the process parameters monitored and the process for determination of yields.

For each purification method or combination of methods used, a tabulation of yields, purity and biological activity shall be provided. Verification of the removal or dilution of product related and non-product related impurities, e.g. processing reagents, endotoxin contaminating cell proteins or nucleic acids, and other residual contaminants shall be included. A standard denominator (e.g. international units) shall be used to facilitate comparison through processing,

concentration, or dilution. If the purified substance is held prior to further processing, a description of the storage conditions and time limits shall be included.

-Stabilisation process (if applicable)
A description shall be provided for any post-purification steps performed to produce a stabilised antigen (e.g. adsorption, addition of stabilisers, addition of preservatives), and the objectives and rationale for performing each process.

A description of precautions taken to monitor environmental monitoring and prevent contamination during these processes shall also be given. If the antigen is held prior to further processing, a description of storage conditions and time limits should be included. Verification of the stability of the vaccine under the conditions described shall be provided under section 2.D.2.

-Provide the criteria for pooling more than one batch (if applicable).
The reuse and/or regeneration of columns and adsorbents and monitoring for residual impurities and leachable reagents should be provided.
Consistency of the manufacturing process for each antigenic component shall be demonstrated by manufacturing at least three, preferably consecutive, batches of vaccine of a size corresponding to that for routine production.

Bulk antigen Container and Closure System

A description of the container and closure system, and its compatibility with the immunogenic substance shall be submitted. The submission shall include detailed information concerning the supplier, address and the results of compatibility, toxicity and biological tests. If the active immunogenic substance is intended to be sterile, evidence of container and closure integrity for the duration of the proposed shelf life shall be provided.

Formulation of the finished product

Include a detailed description of the further manufacturing process flow of the formulated bulk up to the filling of the finished product. This should include the sterilisation operations, aseptic processing procedures, filling, lyophilization (if applicable), and packaging

2.C Control of Starting Materials

A list of all starting materials including culture media, buffers, resins for peptide synthesis, chemicals used in the manufacture of the immunogenic substance and their specifications or reference to official compendia shall be provided. For purchased starting materials, representative certificates of analysis from the supplier(s) and/or manufacturer's acceptance criteria shall be provided.

2.C.1 Starting materials listed in pharmacopoeias

2.C.2 Starting materials not listed in pharmacopoeias

2.C.2.1 Starting materials of non-biological origin
2.C.2.2 Starting materials of biological origin

2.C.2.2.1 Cell seed materials

General Requirements
If a virus can be grown efficaciously on cell cultures based on a seed lot system of established cell lines, no mammalian primary cells should be used. Permanently infected cells shall comply with the appropriate requirements described below. The cells must be shown to be infected only with the agent stated.

2.C.2.2.1.1 Requirements for Cell Lines

Cell seed materials used in manufacture shall normally be produced according to a Seed Lot System. Each Master Cell Seed (MCS) shall be assigned a specific code for identification purposes. The MCS shall be stored in aliquots at -70 °C or lower. Production of vaccine shall not normally be undertaken on cells further than 20 passages from the MCS.

Where suspension cultures are used, an increase in cell numbers equivalent to approximately three population doublings should be considered equivalent to one passage.

If cells beyond this passage level are to be used for production, the applicant should demonstrate, by validation or further testing, that the production cells are essentially similar to the MCS with regard to their biological characteristics and purity and that use of such cells has no deleterious effect on vaccine production.

The history of the cell line must be known in detail and recorded in writing (e.g. origin, number of passages and media used for their multiplication, storage conditions).

The manufacturer must describe the method of preserving and using the cells, including details of how it is ensured that the maximum number of passages permitted is not exceeded during product manufacture. A sufficient number of MCS and Working Cell Seed (WCS) must be kept available for testing by the licensing authorities.

The checks described below should be carried out on a culture of the MCS and WCS or on cells from the WCS at the highest passage level used for production (see Table 1) and derived from a homogeneous representative sample. The representative nature of this sample must be proven.

Table 2: Stages of cell culture at which testing shall be carried out

	MCS	WCS	Cells from WCS at highest passage level
General microscopy	+	+	+
Bacteria/fungi	+	+	-
Mycoplasma	+	+	-
Viruses	+	+	-
Identification of species	+	-	+
Karyology	+	-	+
Tumourigenicity	+	-	-

2.C.2.2.1.1.1 Extraneous contaminants

2.C.2.2.1.1.1.1 General

The cells must be checked for their appearance under the microscope, for their rate of growth and for other factors which will provide information on the state of health of the cells.

2.C.2.2.1.1.1.2 Bacteria and fungi

The cells must be checked for contamination with bacteria or fungi. Contaminated cells must be discarded.

2.C.2.2.1.1.1.3 Mycoplasma

The cells must be checked for freedom from mycoplasma and pass the test for freedom from mycoplasma.

2.C.2.2.1.1.1.4 Viruses

The cells must not be contaminated by viruses and the checks must be performed in the following manner:
The monolayers tested must be at least 70 cm^2, prepared and maintained using medium and additives, and grown under similar conditions to those used for the preparation of the biological product. The monolayers must be maintained in culture for a total of at least 28 days. Subcultures should be made at 7-days intervals, unless the cells do not survive for this length of time, when the subcultures should be made on the latest day possible. Sufficient cells, in suitable containers, must be produced for the final subculture to carry out the tests specified below. The monolayers must be examined regularly throughout the incubation period for the possible presence of cytopathic effects (CPE) and at the end of the observation period for CPE,

haemadsorbent viruses and specific viruses by immunofluorescence and other appropriate tests as indicated below.

2.C.2.2.1.1.1.4.1 Detection of cytopathic viruses

Two monolayers of at least 6 cm^2 each must be stained with an appropriate cytological stain. Examine the entire area of each stained monolayer for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality which might be attributable to a contaminant.

2.C.2.2.1.1.1.4.2 Detection of haemadsorbent viruses

Monolayers totalling at least 70 cm^2 must be washed several times with an appropriate buffer and a sufficient volume of a suspension of appropriate red blood cells added to cover the surface of the monolayer evenly. After different incubation times examine cells for the presence of haemadsorption.

2.C.2.2.1.1.1.4.3 Detection of specified viruses

Tests should be carried out for freedom of contaminants specific for the species or origin of the cell line and for the species for which the product is intended. Sufficient cells on appropriate supports must be prepared to carry out tests for the agents specified. Appropriate positive controls must be included in each test. The cells are subjected to appropriate tests using fluorescein-conjugated antibodies or similar reagents.

2.C.2.2.1.1.1.4.4 Tests in other cell cultures

Monolayers totalling at least 140 cm^2 are required. The cells must be frozen and thawed at least 3 times and then centrifuged to remove cellular debris. Inoculate aliquots onto the following cells at any time up to 70% confluency:

– Primary cells of the source species
– Cells sensitive to viruses pathogenic for the species for which the vaccine is intended
– Cells sensitive to pestiviruses

The inoculated cells must be maintained in culture for at least 7 days, after which freeze-thawed extracts should be prepared as above, and inoculated onto sufficient fresh cultures of the same cell types to allow for the testing as described below. The cells are incubated for at least a further 7 days. All cultures must be regularly examined for the presence of any cytopathic changes indicative of living organisms. At the end of this period of 14 days, the inoculated cells must be subjected to the following checks:

– Freedom from cytopathic and haemadsorbent organisms must be tested for, using the methods specified in paragraphs *2.C.2.2.1.1.1.4.1* and *2.C.2.2.1.1.1.4.2*
– Relevant substrates are tested for the absence of pestiviruses and other specific contaminants by immunofluorescence as indicated in *2.C.2.2.1.1.1.4.3*

2.C.2.2.1.1.2 Identification of species

It must be shown that the MCS and the cells from the WCS at the highest passage level used for production come from the species of origin specified by the manufacturer. This must be demonstrated by one validated method. When a fluorescence test is carried out and the corresponding serum to the species of origin of cells is used and shows that all the tested cells are fluorescent, it is not necessary to carry out other tests with reagents able to detect contamination by cells of other species.

2.C.2.2.1.1.3 Karyology

The cell lines used must be examined in the following manner:
A minimum of 50 cells undergoing mitosis must be examined in the MCS and a passage level at least that of the highest to be used in production. Any chromosomal marker present in the MCS must also be found in the high passage cells. The modal number of chromosomes in these cells must not be more than 15% higher than that of the MCS. The karyotypes must be identical. If the modal number exceeds the level stated, the chromosomal markers are not found in the WCS cells or the karyotype differs, the cell line may not be used for the manufacture of biological products.

2.C.2.2.1.1.4 Tumourigenicity

The potential risk of a cell line for the target species should be evaluated and, if necessary, tests should be carried out.

2.C.2.2.1.2 Requirements for primary cells.

For most of the mammalian vaccines the use of primary cells is not acceptable for the manufacture of vaccines. If a vaccine has to be produced on primary cells, they should be obtained from a specific pathogen free herd or flock with complete protection from introduction of diseases (e.g. disease barriers, filters on air inlets, no new animals introduced without appropriate quarantine). In the case of chicken flocks, these should comply with the requirements of the European Pharmacopoeia monograph for SPF chickens. For all other animals and species of birds, the herd or flock must be shown to be free from appropriate pathogens. All the breeding stock in the herd of flock intended to be used to produce primary cells for vaccine manufacture must be subject to a suitable regime such as regular serological checks carried out at least twice a year and two supplementary serological examinations performed in 15% of the breeding stock in the herd between the two checks mentioned above. Wherever possible, particularly for mammalian cells, a seed lot system should be used with, for example, MCS formed from less than 5 passages, the WCS being no more than 5 passages from the initial preparation of the cell suspension from the animal tissues. Each MCS, WCS and cells of the highest passage of primary cells must be checked in accordance with Table 2 and the procedure described below. The sample tested will cover all the sources of cells used for the manufacture of the batch. No batches of vaccine manufactured using the cells may be marketed if any one of the checks performed produces unsatisfactory results.

Table 3: Stages of primary cell culture at which testing shall be carried out

	MCS	WCS	Cells from WCS at highest passage level
General microscopy	+	+	+
Bacteria/fungi	+	+	–
Mycoplasma	+	+	–
Viruses	+	+	–
Identification of species	+	–	–

2.C.2.2.1.2.1 Extraneous contaminants

See sections *2.C.2.2.1.1.1* to *2.C. 2.2.1.1.1.4.3* above.

2.C.2.2.1.2.2 Tests in other cell cultures

Monolayers totalling at least 140 cm^2 are required. The cells must be frozen and thawed at least 3 times and then centrifuged to remove cellular debris. Inoculate aliquots onto the following cells at any time up to 70% confluency:

– Cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
– Cells sensitive to pestiviruses.

The inoculated cells must be maintained in culture for at least 7 days, after which freeze-thawed extracts should be prepared as above, and inoculated onto sufficient fresh cultures of the same cell types to allow for the testing as described below. The cells are incubated for at least a further 7 days.

All cultures must be regularly examined for the presence of any cytopathic changes indicative of living organisms. At the end of this period of 14 days, the inoculated cells must be subjected to the following checks:

– Freedom from cytopathic and haemadsorbent organisms must be tested for using the methods specified in paragraphs *2.C.2.2.1.1.1.4.1* and *2.C.2.2.1.1.1.4.2*
– Relevant substrates are tested for the absence of pestiviruses and other specific contaminants by immunofluorescence as indicated in *2.C.2.2.1.1.1.4.3*

2.C.2.2.1.2.3 Identification of species

It must be shown that the MCS comes from the species or origin specified by the manufacturer (see Table 2). This must be demonstrated by one validated method. When a fluorescence test is carried out and the corresponding serum to the species or origin of cells is used and shows that all the tested cells are fluorescent, it is not necessary to carry out other tests with reagents able to detect contamination by cells of other species.

2.C.2.2.1.2.4 Requirements for embryonated eggs

Embryonated eggs must be obtained from an SPF flock.

2.C.2.2.1.2.5 Requirements for animals

Animals must be free from specific pathogens, as appropriate to the source species and the target animal.

2. C.2.2.2 Seed Materials

2.C.2.2.2.1 Master seeds

2.C.2.2.2.1.1 Virus seed

2.C.2.2.2.1.1.1General requirements
Viruses used in manufacture shall be derived from a Seed Lot System. Each Master Seed Virus (MSV) shall be tested as described below.

A record of the origin, passage history (including purification and characterisation procedures) and storage conditions shall be maintained for each Seed Lot. Each MSV shall be assigned a specific code for identification purposes. The MSV shall normally be stored in Aliquots at -70°C or lower if it is in liquid form or at -20°C or lower if in a lyophilised form. Production of vaccine shall not normally be undertaken using virus more than 5 passages from the MSV.

In the tests described in sections *2.C.2.2.2.1.1.3, 2.C.2.2.2.1.1.4* and *2.C.2.2.2.1.1.5* below, the organisms used shall not normally be more than 5 passages from the MSV at the start of the tests unless otherwise indicated.

Where the MSV is contained within a permanently infected MCS, the following tests shall be carried out on an appropriate volume of virus from disrupted MCS. Where relevant tests have been carried out on disrupted cells to validate the suitability of the MCS, these tests need not be repeated.

2.C.2.2.2.1.1.2 Propagation

The MSV and all subsequent passages shall be propagated on cells, on embryonated eggs or in animals which have been shown to be suitable for vaccine production. All such propagations shall only involve substance of animal origin that meet the requirements of section 1.1 of the Adopted guidelines "General requirements for the production and control of live mammalian

bacterial and viral immunological products for veterinary use" (WC500004651) and "General requirements for the production and control of inactivated mammalian bacterial and viral immunological products for veterinary use" (WC500004652), effective date September 1992.

2.C.2.2.2.1.1.3 Identity

The MSV shall be shown to contain only the virus stated. A suitable method shall be provided to identify the vaccine strain and to distinguish it as far as possible from related strains.

2.C.2.2.2.1.1.4 Sterility and mycoplasma

The MSV shall pass the tests for sterility and freedom from mycoplasma.

2.C.2.2.2.1.1.5 Extraneous agents

Serum containing a high level of neutralising antibody to the virus of the Seed Lot shall be prepared, using antigen that is not derived from any passage level of the virus isolate giving rise to the MSV. Where it is not possible to prepare such a serum, other methods may be used to remove selectively the virus of a seed lot.

Sera shall be prepared on a batch basis. Each batch shall be shown to be free of antibodies to potential contaminants of the seed virus. Each batch shall be shown to be free of any non-specific inhibition effects on the ability of viruses to infect and propagate within cells (or eggs – if applicable). Each batch shall be treated at 56 °C for 30 minutes to inactivate complement.

Using a minimum amount of serum prepared as above, a sample of the MSV shall be treated so that all the vaccine is neutralised or removed. The final virus/serum mixture shall contain at least the virus content of 10 dose of vaccine per ml if possible. The mixture should then be tested for freedom from extraneous agents as follows.

The mixture shall be inoculated onto cultures of at least 70 cm^2 of the required cell types. The cultures may be inoculated at any stage of growth up to 70% confluency. At least one monolayer of each type must be retained as a control. The cultures must be monitored daily for a week. At the end of this period the cultures are freeze-thawed 3 times, centrifuged to remove cell debris and reinoculated onto the same cell type as above. This is repeated twice. The final passage must produce sufficient cells in appropriate vessels to carry out the tests below.

Cytopathic and haemadsorbing agents are tested for using the methods described in paragraphs *2.C.2.2.1.1.1.4.1* and *2.C.2.2.1.1.1.4.2*. Techniques such as immunofluorescence should be used for detection of specific contaminants as described in paragraphs *2.C. 2.2.1.1.1.4.3*. The MSV is inoculated onto:

– Primary cells of the species of origin of the virus;
– Cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
– Cells sensitive to pestiviruses.

If the MSV is shown to contain living organisms of any kind, other than virus of the species and strain stated, then it is unsuitable for vaccine production.

2.C.2.2.2.1.2 Bacterial seed

2.C.2.2.2.1.2.1 General requirements

The bacteria used in the vaccine shall be stated by genus and species (and varieties where appropriate).

The origin, date of isolation and designation of the bacterial strains used shall be given, and details provided, where possible, of the passage history, including details of the media used at each stage.

Bacteria used in manufacture shall be derived from a Seed Lot System wherever possible. Each Master Seed Lot, (henceforth known as Seed Lot) shall be tested as described below.

A record of the origin, passage history (including purification and characterisation procedures) and storage conditions shall be maintained for each Seed Lot. Each Seed Lot shall be assigned a specific code for identification purposes.

2.C. 2.2.2.1.2.2 Identity and purity

Each Seed Lot shall be shown to contain only the species and strain of bacterium stated. A brief description of the method of identifying each strain by biochemical, serological and morphological characteristics and distinguishing it as far as possible from related strains shall be provided, as shall also the methods of determining the purity of the strain. If the Seed Lot is shown to contain living organisms of any kind other than the species and strain stated, then it is unsuitable for vaccine production.

2.C.2.2.2.1.2.3 Seed lot requirements

The minimum and maximum number of subcultures of each Seed Lot prior to the production stage shall be specified. The methods used for the preparation of seed cultures, preparation of suspensions for seeding, techniques for inoculation of seeds, titre and concentration of inocula and the media used shall be described. It shall be demonstrated that the characteristics of the seed material (e.g. dissociation or antigenicity) are not changed by these subcultures.

The conditions under which each seed lot is stored shall be described.

2.C.2.2.2.1.3 Samples

Samples of all seed materials, reagents, in-process materials and finished product shall be supplied to the competent authorities, on request.

2.C.2.2.2.2 Working seed

Working seed shall be derived from one or more container of Master seed. Working Seed shall be characterized in the same way as working cell bank (WCB). Details on characterization of working seed is as detailed in section 2.H.4.

2.C.2.2.3 Other substances of animal origin

All other substances, used in vaccine production shall be prepared in such a way as to prevent contamination of the vaccine with any living organism or toxin.

2.C.3 Minimising the risk of TSE

Biological starting materials should be characterized sufficiently to ensure that they do not contaminate the final product with extraneous infectious organisms, such as transmissible spongiform encephalopathies (TSEs). For a substance to be considered free of a contaminant, assay should demonstrate, at a predefined level of sensitivity, that a certain quantity of the substance is free of that contaminant. Alternatively, a validated process that is known to remove a contaminant to a defined level may be used to demonstrate the absence of that contaminant. If the contaminant is known to be present in the seed cell material or viral seed, then results to demonstrate that the production process is sufficiently robust to eliminate or inactivate the agent with an appropriate margin of safety should be described.

Documentation to demonstrate that the starting materials and the manufacturing of the veterinary vaccine is in compliance with the requirements of the Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, as well as with the requirements of the corresponding monograph of the European Pharmacopoeia shall be supplied. Certificates of Suitability issued by the European Directorate for the Quality of Medicines and Health Care, with reference to the relevant monograph of the European Pharmacopoeia, may be used to demonstrate compliance

2.C.4 Media preparation

Details of methods of preparation and sterilisation of all media must be provided. Culture media must be stored at the specified temperature, under specified conditions and for no longer than the applicable shelf life. Quality control tests should be carried out to ensure that the performance characteristics of the medium are within specification.

2.D In-process control tests

A description of all analytical testing performed to characterise the active immunogenic substance with respect to identity, quantity and stability with their test results should be presented in either tabular form, legible copies of chromatograms or spectra, photographs of gels or immunoblots, actual histograms of cytometric analysis or other appropriate formats.

Data should be well organised and fully indexed to enable easy access. Results for quantitative assays should be presented as actual data not generally as "Pass" or "Fail".

-Process Validation
A summary report, including protocols and results shall be provided in the Appendix to Part 2 for the validation studies of each critical process or factor that affects active immunogenic substance specifications. The validation study reports that have been subjected to statistical rigor shall demonstrate the variability in each process as it relates to final specifications and quality.

-Control of Bio-burden
For any process, which is not intended to be sterile, documentation of the control of extraneous bioburden by a tabulation of in- process testing for bioburden shall be provided.

2.E Control Tests on the Finished Product

Detailed information on finished product tests performed on each batch, including the batch release specification, must be provided. The following information shall be provided:

(a) Appearance
A qualitative statement describing the physical state (lyophilized solid, powder, liquid) and colour and clarity of the Veterinary vaccine.

(b) Identity
The method used to establish the identity of the vaccine should be described. The description should include an evaluation of specificity and sensitivity of the method.

(c) Purity/sterility
Include information on the purity or sterility of the Veterinary vaccine.

(d) Safety
Provide results of the batch safety tests performed in the target animal species.

(e) Potency/Titre
A description of the potency assay for the Veterinary vaccine should be provided. Information shall be submitted on the sensitivity, specificity, and variability of the assay including the data from the material used to prepare clinical lots which were used to set the acceptance limits for the assay.

(f) Chemical and Physical tests
Provide information on the chemical and physical tests carried out on the finished Veterinary vaccine. These shall include: pH and, if applicable, adjuvant, preservative, residual humidity, viscosity, emulsion, residual inactivant, etc.

(g) Sampling procedures (add information)
The sampling procedures for monitoring a batch of veterinary vaccine shall be included.

(h) Specifications and methods

A description of all test methods selected to assure the identity, purity, titre /or potency, as well as the lot-to-lot consistency of the finished product and the specifications used for the immunogenic product shall be submitted. Certificates of analysis and analytical results for at least three consecutive batches shall be provided.

(i) Validation results

The results of studies validating the specificity, sensitivity, and variability of each method used for release testing shall be provided. Where applicable this shall include descriptions of reference standards and their validation. For analytical methods in compendial sources, the appropriate citations shall be provided

2.F Batch to batch consistency

Provide a table of results from three consecutive batches,

Provide the manufacturing records of these three batches in the Appendix to Part 2.

2.G Stability

2.G.1 Stability of the Final Product

Evidence shall be provided to demonstrate that the product is stable for the proposed shelf life period under the storage conditions described on the label. The ultimate proposed shelf life should be stated.

Stability data should be provided for at least three representative consecutive batches stored in the final container. The three consecutive production runs may be carried out on a pilot scale (10% of full scale), providing this mimics the full-scale production method described in the application, or manufacturing scale (the largest scale validated and proposed for registration for commercial use). The storage temperature should be stated together with the results of tests on the batches. A plan for on-going stability studies should be provided indicating the batch numbers of the batches on test and the time points when testing is planned.

Examples of stability-indicating tests to be performed:

1. Sterility at time 0 and end of shelf life
2. Potency/virus titre/bacterial counts
3. Physical and chemical tests, as appropriate, such as:
    - Moisture content of lyophilised vaccines (VICH GL26).
    - Tests to quantify the adjuvant.
    - Oil adjuvanted vaccine shall be tested for viscosity by a suitable method.
    - The stability of the emulsion shall be demonstrated.

- Quantitative assay of any preservatives. For multi-dose presentations, when a preservative is included in the vaccine, preservative efficacy should also be studied at the minimum and maximum time points to Ph. Eur. 5.1.3 and at the lower preservative limit in the end of shelf life specification if there is a range.
  Note: A preservative may only be included in a single dose vial if it can be shown that the single dose vial is filled from the same bulk blended vaccine as a multi-dose container.
- The pH of liquid products and diluents shall be measured and shown to be within the limits set for the product.

4. Target animal safety testing: for conventional vaccines it may be acceptable to omit the target animal safety test at each shelf life testing point.

Additional Notes:
A short shelf life will be granted, if necessary, while evidence of stability is collected.

The shelf life starts at the time of the first titration (live vaccines) or potency test. For example, for *in vivo* potency tests the shelf life starts from the date of the first administration of the vaccine to the species in which the potency test is carried out.

For vaccines stored by the manufacturer at a temperature lower than that stated on the label, the stability for the entire storage period should be demonstrated. The expiry date is then calculated from the date that the vaccine is stored under the conditions stated on the label.

2.G.2 In-use shelf life

Stability-indicating tests should be provided on at least 2 different batches to support an in-use shelf life. Target animal safety testing should not normally be required.

2.G.2.1 Shelf-life after first opening the container

Generally, an in-use shelf life after first opening should not exceed 8-10 hrs.
For live vaccines an in-use shelf life of 8-10 hours must be supported by virus/bacterial titration data.
For inactivated vaccines omission of the potency test at the end of the in-use shelf life can be justified if the potency test is an *in-vivo* test.

2.G.2.2 Shelf-life after dilution or reconstitution

The shelf life after reconstitution according to the directions should not exceed 10 hours. The product must be reconstituted with the approved diluents and in line with the recommendations. The shelf life after reconstitution must be supported by virus/bacterial titration or potency data. No losses of titre or potency should be observed. For inactivated vaccines omission of the potency test at the end of the in-use shelf life can be justified if the potency test is an *in-vivo* test.

2.G.2.3 Extended in-use shelf life:

A CVMP guideline (EMEA/CVMP/IWP/250147/2008) on data requirements to support in-use stability claims for veterinary vaccines is available. http://www.ema.europa.eu/pdfs/vet/iwp/25014708enfin.pdf. The guideline places emphasis on conducting the in-use stability study mimicking the conditions of use of the vaccine in the field.

Note: For guidance on "Stability testing of Biotechnological Veterinary Medicinal Products" refer to VICH GL 17 (CVMP/VICH/501/99) found at http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

2.H Other Information

2.H.1 Synthetic Peptides

The detail of the peptide synthesis including purification procedures shall be provided.

2.H.2 Conjugates and Modified Immunogenic Substances

This section of the guidance refers to immunogenic substances derived from another immunogenic substance or intermediate through chemical or enzymatic modification, e.g. conjugation of an immunogen to a carrier molecule, enzymatic or chemical cleavage and purification of the non-toxic subunit of a toxin, or derivatisation. The modification may change the fundamental immunogenicity, toxicity, stability or pharmacokinetics of the source immunogenic substance. The derived immunogenic substance may include linking moieties and new antigenic epitopes.

2.H.2.1 Manufacturing procedure

This section should provide a detailed description of:

The specifications and acceptance criteria, for the native immunogenic substance starting materials, which assure suitability for conjugation or modification;

The conditions of all reactions and/or syntheses used to produce a semi-synthetic conjugated molecule, derivatised molecule, or subunit, including intermediate forms of the reactants and immunogenic substance; also include the process parameters which are monitored, in-process controls, testing for identity and biologic activity, and any post-purification steps performed to produce a stabilised derived immunogenic substance.

The application should include a description of the methods and equipment used for separation of unreacted materials and reagents from the conjugate, derivative, or subunit, and a rationale for the choice of methods.

2.H.2.2 Specification

Specifications should be provided for each modified immunogenic substance, including identity, purity, potency, physical-chemical measurements, and measures of stability. If test results for the derived substance will be reported for final release of the immunogenic product a validation report, to include estimates of variability and upper and lower limits, should be provided for each specification. Specifications should include the amount of unreacted starting materials and process reagents unless their removal has been validated.

2.H.3 Guidance for genetic constructs and recombinant cell lines

For recombinant DNA (rDNA) derived products and rDNA-modified cell substrates, detailed information shall be provided regarding the host cells and the source and function of the component parts of the recombinant gene construct.

2.H.3.1 Host cells

A description of the source, relevant phenotype, and genotype shall be provided for the host cell used to construct the biological production system. The results of the characterization of the host cell for phenotypic and genotypic markers including those that will be monitored for cell stability, purity and selection shall be included.

2.H.3.2 Gene construct

A detailed description of the gene, which was introduced into, the host cells, including both the cell type and origin of the source material shall be provided. A description of the method(s) used to prepare the gene construct and a restriction enzyme digestion map of the construct shall be included.

The complete nucleotide sequence of the coding region and regulatory elements of the expression construct, with translated amino acid sequence shall be provided including annotation designating all important sequence features.

2.H.3.3 Vector

Detailed information regarding the vector and genetic elements shall be provided, including description of the source and function of the component parts of the vector e.g. origins of replication, antibiotic resistance genes, promoters, and enhancers. A restriction enzyme digestion map indicating at least those sites used in construction of the vector shall be provided. Critical genetic markers for the characterization of the production cells shall also be indicated.

2.H.3.4 Final gene construct

A detailed description shall be provided of the cloning process, which resulted in the final recombinant gene construct. The information shall include a step-by-step description of the assembly of the gene fragments and vector or other genetic elements to form the final gene construct. A restriction enzyme digestion map indicating at least those sites used in constructions of the final product construct shall be provided.

2.H.3.5 Cloning and establishment of the recombinant cell lines

Depending on the methods to be utilized to transfer a final gene construct or isolated gene fragments into its host, the mechanism of transfer, copy number, and the physical state of the final construct inside the host cell (i.e. integrated or extra chromosomal) shall be provided. In addition, the amplification of the gene construct, if applicable, selection of the recombinant cell clone and establishment of the seed shall be completely described.

2.H.4 Cell banks

A description of the cell bank procedures used shall be provided including:

a) The cell bank system used
b) The size of the cell banks
c) The container and closure system used
d) A detailed description of the methods, reagents and media used for preparation of the cell banks
e) The conditions employed for cryopreservation and storage
f) In-process control(s) and
g) Storage conditions
h) A description shall be provided for the procedures used to avoid microbial contamination and cross-contamination by other cell types present in the facility, and the procedures that allow the banked cells to be traced.

2.H.4.1 Master Cell Bank (MCB)

A complete history and characterization of the Master Cell Bank (MCB) shall be provided, including, as appropriate for the given cells:

a) The biological or chemical method used to derive the cell bank
b) Biochemistry (cell surface markers, isoenzyme analysis, specific protein or mRNA, etc.), Specific identifying characteristics (morphology, serotype etc.)
c) Karyology and tumorigenicity
d) Virulence markers
e) Genetic markers
f) Purity of culture and
g) Media and components (e.g. serum)

2.H.4.2 Working Cell Bank (WCB)

This section shall also contain a description of the procedures used to derive a WCB from the MCB. The description should include the identification system used for the WCB as well as the procedures for storage and cataloguing of the WCB. The assays used for qualification and characterization of each new WCB shall be included with the results of those assays for the WCB currently in use. If applicable, a description of animal passage of the WCB performed to assure the presence of virulence factors, which are protective antigens, shall be supplied.

2.H.4.3 Production Cells

For r-DNA derived immunogenic substances, a detailed description of the characterization of the Production cells that demonstrates that the biological production system is consistent during growth shall be provided. The results of the analysis of the Production cells for phenotypic or genotypic markers to confirm identity and purity shall be included. This section should also contain the results of testing supporting the freedom of the Production cells from contamination by adventitious agents. The results of restriction enzyme analysis of the gene constructs in the cells shall be submitted.

Detailed information on the characterization and testing of banked cell substrates shall be submitted. This shall include the results of testing to confirm the identity, purity and suitability of the cell substrate for manufacturing use.

2.H.4.4 Cell Growth and Harvesting

This section shall contain a description of each of the following manufacturing processes, as appropriate. The description should contain sufficient detail to support the consistency of manufacture of the immunogenic substance.

2.H.4.5 Propagation

This section shall contain description of:

a) Each step in propagation from retrieval of the WCB to culture harvest (stages of growth)
b) The media used at each step (including water quality) with details of their preparation and sterilization
c) The inoculation and growth of initial and sub-cultures, including volumes, time and temperatures of incubation(s)
d) How transfers are performed
e) Precautions taken to control contamination
f) In-process testing which determines inoculation of the main culture system
g) In-process testing to ensure freedom from adventitious agents, including tests on culture cells, if applicable.
h) The nature of the main culture system including operating conditions and control parameters (e.g. temperature of incubation, static vs. agitated, aerobic vs. anaerobic, culture vessels vs. fermenter, volume of fermenter or number and volume of culture vessels)
i) The parallel control cell cultures, if applicable, including number and volume of culture vessels

j) Induction of antigen, if applicable
k) The use of antibiotics in the medium and rationale, if applicable

2.H.4.6 Harvest

A description of the method(s) used for separation of crude substance from the propagation system (precipitation, centrifugation, filtration etc.) shall be provided. Brief description shall be given for the following:

a) The process parameters monitored
b) The criteria for harvesting
c) The determination of yields and
d) The criteria for pooling more than one harvest, if applicable
e) A description of the procedures used to monitor bioburden (including acceptance limits) or sterility shall be included. If the harvested crude immunogenic substance is held prior to further processing, a description of storage conditions and time limits shall be provided.

PART 3: SAFETY

Reports of laboratory tests and field trials performed to demonstrate all aspects of safety of the product during use, together with the conclusions, should be provided.

The reports relating to the laboratory tests and field trials should be written using the sequence of headings below:

1) Title of the test, with reference number
2) Introduction including a statement of the aims of the test study
3) Reference to relevant monographs
4) Name(s) and business address (es) of key personnel and location of the research institute involved in the study
5) Dates of start and end of the test or study
6) Summary
7) Material and methods
8) Results
9) Discussion
10) Conclusion

3.A Laboratory Tests

For guidance on how to design and monitor these studies refer to CVMP/VICH/359665/2005, **VICH GL44**: "Target animal safety for veterinary live and inactivated vaccines" found at http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

3.A.1 Safety of a single dose

The immunological veterinary medicinal product shall be administered at the recommended dosage and by the recommended route of administration to each species in which it is intended to be used. Monitor the animals daily for 14 days, observing and recording objective criteria such as rectal temperature, injection site reaction and effect on performance.

3.A.2 Safety of an overdose

The veterinary vaccine shall be administered at an overdose (normally 10 times the recommended dose for live vaccines and 2 times for inactivated vaccines) by the recommended route of administration to each species in which it is intended to be used. Monitor the animals daily for 14 days, observing and recording objective criteria such as rectal temperature, injection site reaction and effect on performance.

3.A.3 Safety of a repeated dose

The veterinary vaccine shall be shown to be safe by considering the number of doses that are likely to be used to vaccinate the animal during its life time. For example, if the vaccination schedule requires a 2 dose primary course followed by a single annual booster, the repeated administration test should consist of 3 separate doses. The doses may be given 2 weeks apart by the recommended route of administration to each species in which it is intended to be used. This study may be run in conjunction with the single dose study. Monitor the animals daily for 14 days after each administration, observing and recording objective criteria such as rectal temperature, injection site reaction and effect on performance.

3.A.4 Other Safety studies, for live vaccines

a) Spread of the vaccine strain
   Study shedding and spread of the vaccine strain from vaccinated to unvaccinated animals and assess the implications of the results.
b) Dissemination in the vaccinated animal
   Conduct studies to demonstrate if the vaccine strain is present in animal secretions or the tissues of the vaccinated animal.
c) Safety of a live, attenuated vaccine from Reversion to Virulence
   For specific guidance on safety of a live, attenuated vaccine from Reversion to Virulent refer to CVMP/VICH/1052/2004, VICH GL41:"Target animal safety: Examination of live veterinary vaccines in target animals for absence of reversion to virulence." Found at http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5
d) Recombination or genomic re-assortment of strains
   Discuss the probability of recombination or genomic re-assortment with field or other strains.

3.B Field Safety

The safety of the veterinary vaccine should be evaluated during field trials. Both safety and efficacy may be assessed during the same trial. Batches used in the trials must be manufactured according to the method described under Part 2 B.
For specific guidance on conducting field safety trials refer to 852/99, "Field trials with veterinary vaccines." Found at
http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

3.C Other Safety issues to be considered

3.C.1 Safety to the user

For specific guidance on safety to the user refer to CVMP/54533/06, adopted guideline: "User safety for immunological veterinary products." Found at

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

3.C.2 Safety to the environment

For specific guidance on safety to the environment refer to CVMP/074/95 "Environmental risk assessment for immunological veterinary products." Found at http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

3.C.3 Safety of residues

Residues studies are not normally required for veterinary vaccines, however the effects of residues of constituents of the vaccine such as adjuvants or live zoonotic organisms used as antigens should be considered if necessary. Propose a withdrawal period if necessary.

3.C.4 Interactions:

The safety of administering the veterinary vaccine at the same time or at the same site as another immunological veterinary medicinal product must be demonstrated if a recommendation for such use is to be made on the SPC.
For specific guidance on the safety for combined vaccines and associations of immunological veterinary medicinal products refer to CVMP/IWP/594618/2010, "Requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs)." Found at
http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

PART 4: EFFICACY

Particulars of tests which have been performed in the target species of animal regarding the efficacy of the vaccine to support the indications for which it will be used; details of the following studies shall be provided.

Immunogenicity efficacy studies (in target species) including:

4.A Laboratory Efficacy

4.A.1 Controlled clinical studies on efficacy (vaccination-challenge studies)

Provide evidence of efficacy under reproducible controlled conditions. Efficacy will normally be demonstrated by administering a challenge infection with a heterologous strain. If protection against challenge infection has been shown to correlate with serology it may be possible to demonstrate efficacy by serological methods.
The batch (es) used in laboratory efficacy studies will be manufactured and tested according to the methods described in Part 2 of the dossier and contain the minimum quantity of antigen permitted for batch release. It will be administered to the target species at the recommended dose by the recommended route of administration.

4.A.2 Compatibility studies

Where relevant provide the following data:

Studies on potential beneficial interactions with other vaccines administered at the same time.

Studies on potential decrease in efficacy when administered at the same time as other vaccine (interference)

Each individual clinical study protocol shall include the following information

1) Identity and qualifications of key personnel involved
2) Location(s) of study
3) Dates of study
4) Design
5) Selection of animals (inclusion, exclusion criteria)
6) Selection of controls
7) Selection of control treatment (if applicable)
8) Number of animals
9) Response variables – end points
10) Minimisation of bias – randomisation, blinding, compliance
11) Treatments given – identity and quality of the investigational and control products used, dosage used, duration of treatment, duration of observation periods, any concurrent treatments and their justification

12) Analytical methods for determining antibodies if serology is applicable as a measure of efficacy
13) Analysis of results including statistical analysis
14) The proposed indication(s) of the product shall be stated.
15) Discussions and conclusions on efficacy and safety

4.B Field Efficacy

The veterinary vaccine should be tested in controlled field trials. The batch(es) used in field trials will be manufactured and tested according to the methods described in Part 2 of the dossier. It will be administered to the target species at the recommended dose by the recommended route of administration.
For specific guidance on conducting field efficacy trials refer to **852/99**, "Field trials with veterinary vaccines." Found at
http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000374.jsp&mid=WC0b01ac058002ddc5

PART 5: Bibliographical references

Reference to literature shall be precise, quoting the author, year of publication and the relevant page(s). Photocopies of relevant literature may be attached.

Appendix 1

1.Glossary

Antigen – a substance that when introduced into the body stimulates the production of an antibody. Antigens include toxins, bacteria, foreign blood cells, and the cells of transplanted organs. Where an antigen is too small to be recognised by the host it may be linked to a carrier for the purposes of inducing antibodies. Such small antigens are known as haptens.

Applicant – the person, persons or company that applies for a Marketing Authorisation or licence to sell a medicinal product. Once the licence is granted, that Applicant becomes the Marketing Authorisation Holder for that particular medicinal product.

Batch – a defined quantity of starting material, packaging material or product processed in one process or series of processes so that it can be expected to be homogenous. To complete certain stages of manufacture, it may be necessary to divide a batch into a number of sub batches, which are further processed in one process or a series of processes, so that each sub batch can be expected to be homogenous.

Excipient –any pharmacologically inert substance used for combining with an active substance to achieve the desired bulk, consistency, etc.

Finished Product –the formulated medicinal product containing the active ingredient(s) and ready for administration either alone or after reconstitution with the relevant diluent.

Master Cell Seed (MCS) – a collection of aliquots of a preparation of cells, for use in the preparation of a product, distributed into containers in a single operation and processed together in such a manner as to ensure uniformity, and processed and stored in such a manner as to ensure stability.

Master Seed (MS) – a collection of aliquots of a preparation, for use in the preparation and testing of a product, distributed into containers in a single operation and processed together in such a manner as to ensure uniformity, and processed and stored in such a manner as to ensure stability**.**

Primary Cell Cultures – cultures of cells, essentially unchanged from those in the animal tissues from which they have been prepared and being no more than 5 *in vitro* passages to production level from the initial preparation from the animal tissue.

Seed Lot System – a system according to which successive batches of product are prepared using the same Master Cell Seed or Master Seed.

Working Cell Seed (WCS) – a collection of aliquots of a preparation of cells, for use in the preparation and testing of a product, consisting of cells of a passage level intermediate between *Master Cell Seed* and those used for production, distributed into containers in a single operation and processed together in such a manner as to ensure uniformity, and processed and stored in such a manner as the ensure stability.

Working Seed Lot – a collection of aliquots of a preparation consisting of a passage level between MS and the last passage, which forms the finished product, for use in the preparation of finished product, distributed into containers in a single operation and processed together in such a manner as to ensure uniformity, and processed and stored in such a manner as to ensure stability.

Vaccine – A preparation of a weakened (attenuated) or killed pathogen, such as a bacterium or virus, or of a portion of the pathogen's structure, that stimulates immune cells to recognize and attack it, especially through the production of antibodies.

APENDIX 2

2. Abbreviations

2.1 Abbreviations used in this Guideline

CVMP:	Committee for Veterinary Medicinal Products
EAC:	East African Community
EMEA:	European Medicines Evaluation Agency (now known as the EMA: European Medicines Agency)
EPC:	End of Production Cells
Hrs:	hours
MCAZ:	Medicine Control Authority of Zimbabwe
MCB:	Master Cell Bank
MCS:	Master Cell Seed
MSV:	Master Seed Virus
PhEur:	European Pharmacopoeia
TSE:	Transmissible Spongiform Encephalopathy
VICH:	The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.
VICH GL:	Guideline of VICH
WCB:	Working Cell Bank
WCS:	Working Cell Seed
WSV:	Working Cell Virus

2.2 Abbreviations to be found in related documents:

ATCvet code:	The Anatomical Therapeutic Chemical code. This is a classification system for veterinary medicinal products. ATCvet, is based on the same main principles as the ATC classification system for drug substances used in human medicine.
BP:	British Pharmacopoeia
9CFR:	Code of Federal Regulations, Title 9, Animals and Animal Products
EMA:	European Medicines Agency, formally known as EMEA, European Medicines Evaluation Agency
GMO:	Genetically Modified Organism
IFAH:	International Federation of Animal Health
INN:	International Non-proprietary Name
IWP:	Immunologicals Working Party, a subgroup of the CVMP in the EU
OIE:	Office International des Épizooties (World Health Organisation for Animal Health)

rDNA:	ribosomal DNA (Deoxyribonucleic acid); it can also mean recombinant DNA which isDNA artificially constructed by insertion of foreign DNA into the DNA of an appropriate organism so that the foreign DNA is replicated along with the host DNA
SPC:	Summary of Product Characteristics
SPF:	Specific Pathogen Free
WHO:	World Health Organisation
USP:	United States Pharmacopoeia

Appendix 3

APPENDIX III: SCREENING CHECK LIST FOR SUBMISSION OF A DOSSIER FOR REGISTRATION OF A VETERINARY VACCINE

Section	DOCUMENTS	Submitted?			
PART 1	ADMINISTRATIVE INFORMATION	Yes	No	Location (Page numbers)	Critical or non-critical
1.A	ADMINISTRATIVE INFORMATION				
1.B	1. B Summary of Product Characteristics (SPC), Primary container label, secondary container text (carton) and Packaging leaflet				NC
1.B.1	SPC				NC
1.B.2	Primary container label secondary container text (carton)				NC
1.B.3	Package insert				NC
PART 2	QUALITY: MANUFACTURE AND CONTROL				
2.A					
	2.A.1 Table of qualitative and quantitative composition				C
	2.A.2 Containers				NC
2.B	2.B Method of Manufacture				C
	2.B.1 Flow chart				C
	2.B.2 Detailed description of manufacture				C
2.C	2.C Control of Starting Materials				
2.C	2.C.1 Starting materials listed in pharmacopoeias				C
	2.C.2 Starting materials not listed in pharmacopoeias				C
	2.C.3 Minimising the risk of TSE				NC
	2.C.4 Media preparation				C
2.D	CONTROL OF DRUG SUBSTANCE				
2.D	2.D In-process control tests				C
	2.E Control Tests on the Finished Product				C
	2.F Batch to batch consistency				C
	2.G Stability				C
	2.G.1 Stability of the Final Product				C
	2.G.2 In-use shelf life				C
PART 3	PART 3: SAFETY				
3.A	3.A Laboratory Tests				C
3.B	3.B Field Safety				C
PART 4:	EFFICACY				
4.A	4.A Laboratory Efficacy				C
4.B	4.B Field Efficacy				C
SAMPLE	Submission of samples				C

Key: NC: Not Critical
C: Critical