GUIDELINE

ON THE TECHNICAL DOCUMENTATION REQUIRED TO BE INCLUDED IN A REGISTRATION DOSSIER FOR AN IMMUNOLOGICAL VETERINARY PRODUCT

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Table of Contents

PART 2: QUALITY: MANUFACTURE AND CONTROL 6
2.A Quantitative and Qualitative Particulars 6
2.A.1 Table of qualitative and quantitative composition 6
2.A.2 Containers 6
2. B Method of Manufacture 6
2.B.1 Flow chart 6
2.B.2 Detailed description of manufacture 5
2.C Control of Starting Materials 6
2.C.2 Starting materials listed in pharmacopoeias 6
2.C.3 Starting materials not listed in pharmacopoeias 6
2.C.4 Minimising the risk of TSE 14
2.C.5 Media preparation 14
2D In-process control tests 14
2E Control Tests on the Finished Product 15
2 F Batch to batch consistency 16
2G Stability 16
2.G.1 Stability of the Final Product 16
2.G.2.In-use shelf life 17
2H Other Information 17
2.H.1 Synthetic Peptides 17
2.H.2 Conjugates and Modified Immunogenic Substances 18
2.H.3 Guidance for genetic constructs and recombinant cell lines 18
2.H.4 Cell banks 19
PART 3: SAFETY 22
3.A Laboratory Tests 22
3.A.1 Safety of a single dose 22
3.A.2 Safety of an overdose 22
3.A.3 Safety of a repeated dose 22
3.A.4.Other Safety studies, for live vaccines 23
3.B Field Safety 23
3.B. Other Safety issues to be considered 23
3.B.1.Safety to the user 23
3.B.2 Safety to the environment 23
3.B.3.Safety of residues 23
3.B.4 Interactions: 24
PART 4: EFFICACY 25
4.A Laboratory Efficacy 25
4.A.1 Controlled clinical studies on efficacy (vaccination-challenge studies) 25
4.A.2 Compatibility studies 25
4.B Field Efficacy 26
PART 5: Bibliographical references 26
Appendix 27

ANNEX I: GL2-V3 ANNOTATED GUIDELINE 1
Introduction 5
PART 2: QUALITY: MANUFACTURE AND CONTROL 6
2.A Quantitative and Qualitative Particulars 6
2.A.1 Table of qualitative and quantitative composition 6
2.A.2. Containers 7
3.B Method of Manufacture 7
2.B.1 Flow chart 7
2.B.2 Detailed description of manufacture 8
2.C Control of Starting Materials 11
2.C.1Starting materials listed in pharmacopoeias 12
2.C.2 Starting materials not listed in pharmacopoeias 12
2.C.3.Minimising the risk of TSE 19
2.C.4 Media preparation 19
2. D In-process control tests 20
2 E Control Tests on the Finished Product 20
2.F Batch to batch consistency 23
2.G Stability 23
2.G.1 Stability of the Final Product 24
2.G.2 In-use shelf life 24
2.H Other Information 25
2.H.1 Synthetic Peptides 25
2.H.2 Conjugates and Modified Immunogenic Substances 25
2.H.3.Guidance for genetic constructs and recombinant cell lines 26
2.H.4.Cell banks 27
PART 3: SAFETY 30
3.A Laboratory Tests 30
3.A.1 Safety of a single dose 30
3.A.2 Safety of an overdose 31
3.A.3.Safety of a repeated dose 31
3.A.4.Other Safety studies, for live vaccines 31
3.B Field Safety 32
3.C. Other Safety issues to be considered 33
3.C.1 Safety to the user 33
3.C.2 Safety to the environment 33
3.C.3 Safety of residues 33
3.C.4.Interactions: 34
PART 4: EFFICACY 35
4.A. Laboratory Efficacy 36
4.A.2. Compatibility studies 36
4.B Field Efficacy 37
PART 5: Bibliographical references 39
2. Abbreviations 41
2.1 Abbreviations used in this Guideline 41
2.2. Abbreviations to be found in related documents: 41

GUIDELINE ON THE TECHNICAL DOCUMENTATION REQUIRED TO BE INCLUDED IN A REGISTRATION DOSSIER FOR AN IMMUNOLOGICAL VETERINARY PRODUCT

Introduction

This document is intended for use by applicants developing immunological veterinary products for registration within the East African Community (EAC). This Guideline GL2 has annex I for the annotated GL2 version 3 for applicants intending to submit application for (Foot and Mouth disease) FMD vaccine.

The information provided in this document is intended to provide guidance to the applicant in generating the appropriate data for inclusion in a registration application dossier[1]. It is intended to facilitate the interpretation and application of the EAC legislation concerning the sale and supply of immunological veterinary products within the East African Community.

This guideline is not legally binding. It is intended for information only. Nevertheless, it represents the harmonised view of the Member States of the EAC.

The East African Community is currently composed of 5 Member States, the Republics of Kenya, Uganda, the United Republic of Tanzania, the Republic of Rwanda and the Republic of Burundi. It was established by a Treaty signed on 30th November 1999 and came into force on 7th July 2000. The original Member States were Kenya, Tanzania, and Uganda. These were joined by the Republics of Rwanda and Burundi when they became full members of the Community in July 2007 and South Sudan which joined in 2016. It is envisaged that other countries may join the EAC in future and some others who are not Member States may wish to recognize and take part in the process of harmonised registration of veterinary vaccines in the EAC.

This guideline provides details about the type of Quality information concerning the Manufacture and Control of veterinary immunologicals 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. The applicant is expected to refer to GL1(Registration dossier structure for an immunological veterinary products) on EAC's website at: https://www.eac.int/documents/category/livestock

In addition to the sections of the dossier covered by this guideline, the applicant is required to complete a harmonised Application Form for inclusion with Part 1 of the dossier. Draft Packaging text and the proposed SPC should be included in the Part 1.

See template on EAC's website at:
https://www.eac.int/documents/category/livestock for the Application Form and Packaging Template.

[1] See Dossier Structure document
https://www.eac.int/documents/category/livestock

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 immunological veterinary product 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 Immunological Veterinary Product

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 immunological veterinary product. 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 bio-burden 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 active immunogenic substance 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 active immunogenic substance 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, lyophilisation (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.2 Starting materials listed in pharmacopoeias

2.C.3 Starting materials not listed in pharmacopoeias

2.C.3.1 Starting materials of non-biological origin
2.C.3.2 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.2.2.2.1.1.1 General 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 the Guideline on requirements for the production and control of immunological

veterinary medicinal products (EMA/CVMP/IWP/206555/2010 Rev.1)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/12/WC500218307.pdf

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.4 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 immunological veterinary product 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 (EMA/410/01 rev.3)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf,

and the Requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products (EMA/CVMP/743/00 Rev.2)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004575.pdfp&mid=WC0b01ac058002ddc5

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.5 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 Immunological Veterinary Product.

(b) Identity
The method used to establish the identity of the IVP 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 Immunological Veterinary Product.

(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 Immunological Veterinary Product 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 Immunological veterinary product. 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 immunological veterinary product 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.

2G 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/ema/index.jsp?curl=pages/regulation/general/general_content_001639.jsp&mid=WC0b01ac058002ddc6. The guideline places emphasis on conducting the in-use stability study by 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

2H 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004553.pdf

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 immunological veterinary product 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 immunological veterinary product shall be shown to be safe by considering the number of doses that are likely to be used to vaccinate the animal during its lifetime. 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 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004552.pdf
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 immunological veterinary product 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004598.pdf

3.B. Other Safety issues to be considered

3.B.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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004574.pdf

3.B.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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004620.pdf

3.B.3.Safety of residues

Residues studies are not normally required for immunological veterinary products, 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.B.4 Interactions:

The safety of administering the immunological veterinary product 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/docs/en_GB/document_library/Scientific_guideline/2013/07/WC500146676.pdf

PART 4: EFFICACY

Particulars of tests which have been performed in the target species of animal regarding the efficacy of the IVP 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 immunological veterinary product 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004598.pdf

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. Glossary

Active Immunogenic substance – the active substance in an immunological medicinal product, e.g. a vaccine, which is included as (one of) the antigen(s) of that formulated immunological medicinal product.

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.

Immunological Veterinary Product – a veterinary medicinal product with an immunological mode of action, i.e. it induces immunity to the active substance(s) which it has been formulated.

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.

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

IVP: immunological veterinary product

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 (International Office of Epizootics)

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

ANNEX I: GL2-V3 ANNOTATED GUIDELINE

GUIDELINE

ON THE TECHNICAL DOCUMENTATION REQUIRED TO BE INCLUDED IN A REGISTRATION DOSSIER FOR AN IMMUNOLOGICAL VETERINARY PRODUCT

Annotated version to assist assessors with evaluation of vaccines against Foot and Mouth Disease in the context of the East African Community Mutual Recognition Procedure (EAC MRP)

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GUIDELINE ON THE TECHNICAL DOCUMENTATION REQUIRED TO BE INCLUDED IN A REGISTRATION DOSSIER FOR AN IMMUNOLOGICAL VETERINARY PRODUCT

Table of Contents

ANNEX I: GL2-V3 ANNOTATED GUIDELINE 1
Introduction 5
PART 2: QUALITY: MANUFACTURE AND CONTROL 6
2.A Quantitative and Qualitative Particulars 6
2.A.1 Table of qualitative and quantitative composition 6
2.A.2. Containers 7
3.B Method of Manufacture 7
2.B.1 Flow chart 7
2.B.2 Detailed description of manufacture 8
2.C Control of Starting Materials 11
2.C.1Starting materials listed in pharmacopoeias 12
2.C.2 Starting materials not listed in pharmacopoeias 12
2.C.3.Minimising the risk of TSE 19
2.C.4 Media preparation 19
2. D In-process control tests 20
2 E Control Tests on the Finished Product 20
2.F Batch to batch consistency 23
2.G Stability 23
2.G.1 Stability of the Final Product 24
2.G.2 In-use shelf life 24
2.H Other Information 25
2.H.1 Synthetic Peptides 25
2.H.2 Conjugates and Modified Immunogenic Substances 25
2.H.3.Guidance for genetic constructs and recombinant cell lines 26
2.H.4.Cell banks 27
PART 3: SAFETY 30
3.A Laboratory Tests 30
3.A.1 Safety of a single dose 30
3.A.2 Safety of an overdose 31
3.A.3.Safety of a repeated dose 31
3.A.4.Other Safety studies, for live vaccines 31
3.B Field Safety 32
3.C. Other Safety issues to be considered 33
3.C.1 Safety to the user 33
3.C.2 Safety to the environment 33
3.C.3 Safety of residues 33
3.C.4.Interactions: 34
PART 4: EFFICACY 35
4.A. Laboratory Efficacy 36
4.A.2. Compatibility studies 36
4.B Field Efficacy 37
PART 5: Bibliographical references 39
2. Abbreviations 41
2.1 Abbreviations used in this Guideline 41
2.2. Abbreviations to be found in related documents: 41

Objective, format and advice to assessors on how to use this annotated guideline when evaluating vaccines against Foot and Mouth Disease (FMD)

- This version of the EAC Guideline 2 (GL2) has been annotated by the addition of text in boxes. The annotations are intended to highlight those parts of the dossier that should be given particular attention when assessing applications for FMD vaccines in member countries of the East African Community. As with all EAC guidelines that are intended to facilitate the EAC Mutual Recognition Procedure, this annotated GL2 may also be useful to other countries in Eastern Africa that are not directly involved in the MRP.
    - The annotations are intended to assist assessors in evaluating compliance of a FMD vaccine with the data requirements defined in the text of GL2 and in the regulatory texts cited in this GL. The annotations are intended to ensure that data are provided and assessed against the standards defined in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (the "OIE Terrestrial Manual").
    - The annotations do not attempt to repeat the requirements cited in GL2 but aim to highlight requirements that are important for the evaluation of FMD vaccines, particularly where these requirements are specific to vaccines against FMD when compared with other inactivated viral vaccines
    - Reference is also made to other regulatory texts (as listed below), such as the European Pharmacopoeia and guidelines of the VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products), either where these are specifically referenced in the existing GL2 or where these texts are helpful in describing one possible approach by which compliance with the OIE Terrestrial Manual might be demonstrated. Assessors should note that manufacturers may choose other approaches to demonstrate compliance with GL2.
    - These annotations are not intended to be exhaustive or to repeat in detail the requirements laid out in other regulatory texts. For this reason, assessors will find it useful to have access to the following texts when evaluating FMD vaccine dossiers and should refer to the original texts that are cited in annotations for the necessary detail.

Continued in next box…………

The key texts are

- The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (the "OIE Terrestrial Manual")
  - Particular requirements for FMD vaccines are described in Section C. 'Requirements for vaccines' of Chapter 3.1.8 Foot and Mouth Disease (Infection with Foot and Mouth Disease Virus)
  - Section 2.3 of the OIE Terrestrial Manual points out that "None of these [disease-specific] chapters should be read in isolation. Each is designed to complement and inform the application of Chapter 1.1.8. 'Principles of Veterinary Vaccine Production' in specific situations."
  - Section 2.3 also states "Further chapters deal with sterility and freedom from contamination of biological materials (Chapter 1.1.9) and the management of vaccine banks (Chapter 1.1.10). In addition, many of the general principles of laboratory management set out in Chapter
  - 'Management of veterinary diagnostic laboratories' are applicable to vaccine production, including such areas as accountability, executive management, infrastructure, human resources and compliance".
  - The OIE Terrestrial Manual also contains Chapter 2.3.3. 'Minimum requirements for the organisation and management of a vaccine manufacturing facility' and Chapter 2.3.4 'Minimum requirements for the Production and Quality Control of Vaccines'. Both of these chapters may be relevant when assessing information provided in the dossier on methods of manufacture and control to ensure compliance with OIE standards. However, compliance with these chapters is more usually assessed as part of an inspection for compliance with Good Manufacturing Practice which is not within scope of these annotations. Assessors are recommended to include any potential evidence for deviations from the standards described in these chapters in their assessment report for subsequent follow-up by the relevant GMP inspectorate.
- Monograph 0063 of the European Pharmacopoeia 'Foot-and-Mouth Disease (Ruminants) Vaccine (Inactivated)'
- Reference is made to other monographs of the European Pharmacopoeia defining standards for the quality of materials used in the production of vaccines and to the system operated by the European Directorate for the Quality of Medicine for issuing certificates of suitability where these are cited in GL2
- Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010 Rev.1)
- Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)
- VICH GL44 "Target animal safety for veterinary live and inactivated vaccines"

The annotations included in this version of GL2 are based on the presentations and outcome from the workshop "Harmonising Evaluation of Foot and Mouth Disease Vaccines in Eastern Africa" which was held online, 10-12 November 2020. The final report of the workshop provides links to separate presentations covering in more detail the particular requirements for demonstrating quality, safety and efficacy of FMD vaccines. Assessors should consult these presentations for more detail on the background and content

GUIDELINE ON THE TECHNICAL DOCUMENTATION REQUIRED TO BE INCLUDED IN A REGISTRATION DOSSIER FOR AN IMMUNOLOGICAL VETERINARY PRODUCT

Introduction

The annotated guideline is intended for use by applicants developing Foot and Mouth Disease (FMD) vaccine for registration within the East African Community (EAC). The annotated guideline GL2-V3 is annexed to GL2-V2 (Annex I : For the applicants intending to submit application for Foot and Mouth disease (FMD) vaccine).

The information provided in this document is intended to provide guidance to the applicant in generating the appropriate data for inclusion in a registration application dossier[1]. It is intended to facilitate the interpretation and application of the EAC legislation concerning the sale and supply of immunological veterinary products within the East African Community.

This guideline is not legally binding. It is intended for information only. Nevertheless, it represents the harmonised view of the Member States of the EAC.

The East African Community is currently composed of 5 Member States, the Republics of Kenya, Uganda, the United Republic of Tanzania, the Republic of Rwanda and the Republic of Burundi. It was established by a Treaty signed on 30th November 1999 and came into force on 7th July 2000. The original Member States were Kenya, Tanzania, and Uganda. These were joined by the Republics of Rwanda and Burundi when they became full members of the Community in July 2007 and South Sudan which joined in 2016. It is envisaged that other countries may join the EAC in future and some others who are not Member States may wish to recognize and take part in the process of harmonised registration of veterinary vaccines in the EAC.

This guideline provides details about the type of Quality information concerning the Manufacture and Control of veterinary immunologicals 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. The applicant is expected to refer to GL1(Registration dossier structure for an immunological veterinary products) on EAC's website at: https://www.eac.int/documents/category/livestock

In addition to the sections of the dossier covered by this guideline, the applicant is required to complete a harmonised Application Form for inclusion with Part 1 of the dossier. Draft Packaging text and the proposed SPC should be included in the Part 1. See template on EAC's website at:
https://www.eac.int/documents/category/livestock for the Application Form and Packaging Template.

[1] See Dossier Structure document
https://www.eac.int/documents/category/livestock

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 immunological veterinary product 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 Immunological Veterinary Product

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

- For FMD vaccines the amount of antigen is usually expressed as the minimum PD50/dose.
- Generally, manufacturers will blend vaccines to contain a target amount (or minimum and maximum amounts) of antigen, as measured by determination of 146S content before addition of adjuvant. Data should be presented in the dossier to demonstrate that batches of vaccine containing the minimum antigen content have at least the minimum potency, in terms of PD50, that is stated on the label and in the table of active ingredients, above.
- Further information on measuring the potency of vaccines, the minimum acceptable potency and the relationship between potency and antigen content may be given under Part 3 Efficacy.
- This section should include details of all strains of FMD virus included in the vaccine. Justification for the choice of vaccine strain should be included here or applicants should cross refer to the section of the dossier where such information can be found (likely to be under Part 3 Efficacy). Applicants should provide evidence that the strains included in the vaccine are likely to provide protection against field strains of FMD virus that are either circulating in the country in which authorisation is sought or that pose a threat to that country. Data and recommendations from international reference laboratories for FMD, such as reports from the FAO/OIE Reference Laboratory for FMD at the Pirbright Institute, are useful to justify the choice of vaccine strain(s). Details of the history, characterisation and quality control of the master seed viruses should be included in Section 2.C.2.2.2.1.1 'Virus seed'. Data from the antigenic and genetic tests that are applied to characterize the virus seed can be useful in justifying the choice of vaccine strain.
- Assessors should cross refer to studies in Part 3 Safety (maximum) and Part 4 Efficacy (minimum) to support the maximum and minimum amounts of antigen that can be incorporated into the vaccine. In the case of multivalent vaccines, the applicant should justify that a vaccine containing the cumulative maximum total amount of antigen permitted (i.e., the total antigenic mass of all antigens in the vaccine) has been shown to be safe.

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.

3.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 immunological veterinary product. 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

> The definitive text describing the special requirements that apply for the manufacture of FMD vaccine is the OIE Manual Chapter 3.1.8, Section 2 'Method of Manufacture'. The Ph. Eur monograph 'Inactivated FMD Vaccines for Ruminants 0063', Section 2 'Production' also provides a useful description of the requirements for production of FMD vaccines.

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

Inactivation

- In the case of FMD vaccines, particular attention should be given to the information provided in Part 2.B.2. of the EAC MRP dossier describing the procedure applied to inactivate the antigen in line with the requirements of Sections 2 and 3 of Part C of the OIE FMD Chapter 3.1.8.
- For most other inactivated viral vaccines, the kinetics of inactivation (i.e. the rate of inactivation) is validated once as part of the development of the process by the manufacturer and documented in the dossier. Confirmation of innocuity (i.e. freedom from live virus) is then performed routinely as an in-process and, in some cases, a final product test. Exceptionally, in the case of FMD vaccines, the kinetics of inactivation should be monitored for each batch of antigen in line with OIE FMD Chapter, Part C, Section 3.1. which states that

  … the inactivation procedure is not considered to be satisfactory unless at least the latter part of the slope of the line is linear and extrapolation indicates that there would be less than one infectious particle per 10^4 litres of liquid preparation at the end of the inactivation period'.
- Linear inactivation kinetics ('first order kinetics') is usually achieved by the use of aziridine based compounds (normally binary ethyleneimine - BEI).
- The use of a second vessel to which the viral harvest is transferred after addition of the inactivant should be confirmed

Key points are that

- the method is fully validated and meets the requirements described in the OIE Manual
- validation includes data from a batch(es) containing the maximum pre-inactivation titre that is specified in the Method of Manufacture
- validation shall be performed for each strain included on the authorisation
- data demonstrating that the kinetics of inactivation are linear and the appropriate end point is reached should be generated for each batch and form part of the batch record

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.

Purification of antigen

Purification of the bulk harvest is an important issue for those FMD vaccines that make claims with respect to DIVA properties. Differentiation of infection from vaccination in the case of FMD vaccines relies on the principle that animals that have been infected generate antibodies against both structural and non-structural proteins (NSP) of the FMD virus. Antibodies against NSP arise due to active replication of the virus in infected animals that results in the production of NSP that are immunogenic. In contrast, animals vaccinated with purified preparations of inactivated FMD virus particles produce antibodies only against the structural proteins of the virus. For a vaccine to have DIVA properties manufacturers will therefore generally include a purification step to remove the NSP that are produced as part of viral replication in culture.

There are no regulatory standards setting approved methods for purification or for certifying that antigens are free of NSP. The manufacturer must therefore justify and validate that the method they apply produces an antigen that does not induce antibodies to NSP. Both the OIE Manual (Chapter 3.1.8. Part C. Section 5.4 'Purity: Testing for antibody to NSP) and the CVMP Position Paper on FMD vaccines (EMEA/CVMP/775/02- FINAL) contain sections describing requirements that manufacturers should meet if claims for DIVA properties are proposed on the summary of product characteristics (SPC) for an FMD vaccine.

Generally, manufacturers should provide data to demonstrate that purified, concentrated antigens are either free from NSP or that NSP are present within acceptable limits.

In addition, as part of registration, manufacturers should demonstrate that vaccination does not induce antibodies to NSP. Typical protocols that may be used to generate sera from vaccinated animals for NSP antibody testing are described in both the OIE and CVMP documents and involve administration on at least two occasions when applied either as a batch test or as part of the information validating the process of manufacture. These two protocols are given as possible examples, other protocols may be used provided the manufacturer can justify that they adequately demonstrate a lack of NSP immunogenicity by the vaccine.

Consideration needs to be given to the DIVA serological test that will be applied to confirm freedom from NSP in vaccinated animals in addition to the vaccination protocol that will be applied to demonstrate that batches of vaccine typical of production do not induce NSP antibodies. The OIE Manual describes serological tests appropriate for detection of NSP antibodies (Part B 'Serological Tests', Section 2.4. 'Nonstructural protein (NSP) antibody tests'.

Where possible, laboratory data should be supplemented by field data demonstrating that the vaccine does not induce NSP antibodies when applied at a population level.

In principle, manufacturers should submit data validating the NSP antibody test on which their claim for DIVA properties is based. In practice, manufacturers will often use commercial NSP tests for this purpose. This is acceptable provided that there is sufficient data available in the public domain for the regulatory authority to be assured that the test applied is fit-for-purpose when applied for the purpose described in the SPC.

-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 bio-burden 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 active immunogenic substance under the conditions described shall be provided under section 2.D.2.

Stability of stored antigen

FMD virus is labile and can be readily degraded by heat and changes in PH. Manufacturers should therefore specify the maximum period of time for which antigens may be stored and provide data to demonstrate that the antigen remains stable for the period specified. Methods for storage and monitoring of concentrated antigens are described in the OIE FMD Chapter, Part C, Section 6. Although these requirements refer specifically to storage of FMD antigens in strategic reserves ("Antigen Banks"), the sections relating to monitoring the quality of stored antigens by measuring the amount of intact 146S particles apply equally to antigens stored by manufacturers prior to blending. Information is also provided in this section on how antigen stocks should be labelled and stored.

-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 active immunogenic substance of a size corresponding to that for routine production.

Bulk antigen Container and Closure System

Pooling of antigen batches
Manufacturers often pool different batches of the same antigen before blending the final product. Information should be provided on the procedure for selecting antigen batches for pooling and how consistency of the antigen prior to blending is ensured.

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, lyophilisation (if applicable), and packaging

2.C Control of Starting Materials

Special considerations for extraneous agent testing applied during the production of FMD vaccines including cell seeds, virus seeds and substances of animal origin
The general requirements that apply to inactivated viral vaccines that are
described in this section of the GL for demonstrating freedom from extraneous agents (EA) apply equally to FMD vaccines. Demonstrating freedom from EA should follow the principles described in Chapter 1.1.9. of the OIE Manual 'Tests for sterility and freedom from contamination of biological materials intended for veterinary use'.
In order to obtain a marketing authorisation for an FMD vaccine for routine use, full compliance with the requirements for freedom from extraneous agents should be provided for all strains included on the authorisation as well as for materials of biological origin and the cell lines used to produce FMD antigens. This will generally require a combination of risk assessment and targeted testing. The concept of 'general' versus 'specific' tests can be taken into account, provided that the ability has been demonstrated of the 'general' tests applied to detect each of the agents for which testing is required and for which specific tests are not applied.
Exceptionally for FMD vaccines, in emergency situations where there is no time to complete full testing of the MSV, provisional acceptance of a new strain onto an existing authorisation may be acceptable on the basis of a risk assessment. This should take into account the origin of the strain and all substances of animal origin that were used in its isolation and propagation; treatments applied during establishment of the Master Seed Virus including the application of organic solvents to inactivate enveloped viruses; the ability of the inactivation procedure applied during routine production using BEI to inactivate potential EA; and the tests applied to detect potential EA in the Master Seed. Generally, the results of testing to demonstrate full compliance with the requirements in this chapter would be required before the new strain could be accepted fully onto the authorisation.

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.1Starting 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.2.2Starting 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.

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

2.C.2.2.1.2.6 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.2.2.2.1.1.1 General 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 the Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010 Rev.1) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/12/WC500218307.pdf

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 immunological veterinary product 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 (EMA/410/01 rev.3)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf,

and the Requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products (EMA/CVMP/743/00 Rev.2)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004575.pdfp&mid=WC0b01ac058002ddc5

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.

This guideline has been superseded by the following notice from the European Commission. Any updates to this notice that are applicable to member countries can be found in the latest version of the European Pharmacopoeia https://www.ema.europa.eu/en/documents/scientific-guideline/minimising-risk- transmitting-animal-spongiform-encephalopathy-agents-human-veterinary- medicinal_en.pdf

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”.

In-process control tests for FMD vaccines

- Part C.3. of the FMD Chapter of the OIE Manual specifies that the following tests should be carried out as in-process controls and provides the specifications that must be met
  Inactivation kinetics, as described in Section 2.B.2. ‘Inactivation’, above
  Innocuity control
- Information on the validation of these tests (fit for purpose) and data from their application on three consecutive vaccine batches (process validation, as specified in Part C.5.5.1 ‘Manufacturing process’) should be provided, including the test methods applied to each batch of each antigen used to formulate the batches of vaccine concerned.
- Data on purity with respect to NSP may not be required as a batch test, provided that sufficient information has been provided in the dossier to demonstrate that the processes applied during manufacture consistently result in an antigen that does not induce antibodies in NSP, as covered in Section 2.B.2 ‘Detailed description of manufacture’, above.

-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:

Control tests for finished FMD vaccines

- Chapter 3.1.18 Section C.4 'Final product batch tests' of the OIE Manual specifies that the following final product tests should be performed by the manufacturer

  - *Innocuity (absence of live virus)*

  The OIE Manual specifies a test for innocuity of the final product involving elution of antigen. Adjuvants and excipients may interfere with the ability of cells to detect live virus requiring the development and validation of suitable techniques for elution of antigen from the formulated product. This can be technically challenging to achieve, and if performed, needs to be fully validated. For these reasons authorities may accept that an innocuity test is not required to be performed on the final product, provided that an innocuity test is performed on the bulk inactivated antigen and all other requirements for consistency of production have been met.

  - *Sterility (absence of bacterial contamination)*

  - *Identity*

  Manufacturers should demonstrate that inactivated antigens from each of the virus(es) specified on the authorisation are present in the final product and no others. Manufacturers may use serological or genetic tests, such as strain-specific PCR, for this purpose.

  - *Purity*

  In the case of FMD vaccines, the OIE Manual uses the term 'purity' in this context to refer to freedom from non-structural proteins, as discussed in Section 2.B2. 'Detailed description of manufacture', above. As discussed above for in-process tests on batches of antigen, a test on the final product for purity with respect to NSP may not be required, provided that sufficient information has been provided in the dossier to demonstrate that the processes applied during manufacture consistently result in an antigen that does not induce antibodies in NSP, as covered in Section 2.B.2, above.

  - *Safety*

  The manufacturer should demonstrate that local and systemic reactions are within accepted limits for each batch of antigen released. Authorities may waive the requirement for target animal batch safety tests provided that manufacturer have adequately demonstrated consistency of production and provided evidence from a sufficient number of production batches to provide assurance of safety, as described in VICH GL50 'Harmonisation of criteria to waive target animal batch safety testing for inactivated vaccines for veterinary use'.

  The abnormal toxicity test involving administration of FMD vaccine to non-target species such as mice and guinea-pigs is no longer considered to add value to the safety evaluation of veterinary vaccines and should not be required as final product safety test.

- *Potency*

This box considers the particular requirements for the batch potency test for FMD vaccines. The relationship between potency and efficacy is discussed further under Part 3 ‘Efficacy’, below.

The definitive test for potency measures in vivo protection of cattle 21 days after vaccination against a standardised challenge. Protocols are described in Chapter 3.1.8 Section C.5.3 ‘Efficacy’ of the OIE Manual for determining potency i.e. estimating the number of 50% protective doses (PD50) or protection against generalized infection (PGP).

Where the manufacturer has not validated a batch potency test, authorities may require that the test described under Efficacy is performed on each batch.

In vitro serological tests may be accepted for the batch release potency test provided that a correlation has been shown between serological titre and protection and a cut-off defined that corresponds with a vaccine of the minimum potency stated in the authorisation. Tests that have been found to be acceptable can be found in the OIE Manual Chapter 3.1.8. Section 4.6.1. ‘Expected Percentage of Protection – EPP’ and in Monograph’ 0063 of the European Pharmacopoeia Section 2-5-4 ‘Batch potency test’.

Validation of the batch potency test and the cut-off applied is important in ensuring that batches released onto the market meet the specification for potency in the authorisation and meet or exceed the minimum potency of 3PD50 defined in the OIE Manual.

Manufacturers may submit a combination of their own study data and data in peer-reviewed publications. Where reference is made to published data the manufacturer should justify their relevance with respect to the proposed batch potency test, particularly in terms of the vaccines tested (e.g. oil, alum, other adjuvant, single vs. multiple vaccination protocol) and the serological test applied (e.g. ELISA, VNT).

As described in the OIE Manual Chapter 3.1.8. Part C. Section 5.3.4. ‘Efficacy in other species’. A successful potency, and batch potency, test in cattle is generally considered to demonstrate the quality of a vaccine sufficiently to justify its use in other species included on the authorisation. For vaccines containing strains with a particular tropism for species other than cattle, such as pigs, efficacy and batch potency should normally be demonstrated in the species indicated on the label. This is likely to require the development and validation of potency, and batch potency, tests in the target species.

Where applications relate to authorisation of a vaccine intended for release in the event of a disease emergency that is formulated from stored antigens (i.e. vaccine from a vaccine bank), Section 7 of the OIE Manual Chapter 3.1.8. Part C. applies which states that:

In situations of extreme urgency and subject to agreement by the National Veterinary Authority, a batch of vaccine may be released before completion of the tests and the determination of potency if a test for sterility has been carried out on the bulk inactivated antigen and all other components of the vaccine and if the tests for safety and the determination of potency have been carried out on a representative batch of vaccine prepared from the same bulk inactivated antigen. In this context, a batch is not considered to be representative unless it has been prepared with not more than the amount of antigen or antigens and with the same formulation as the batch to be released.

(a) Appearance

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

(b) Identity

The method used to establish the identity of the IVP 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 Immunological Veterinary Product.

(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 Immunological Veterinary Product 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 Immunological veterinary product. 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 immunological veterinary product 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

As discussed above under Section 2.B.2 FMD virus is highly labile and therefore data to support the stability of the vaccine throughout the full duration of the shelf life is important.
Manufacturers should repeat the final product tests at regular intervals and a full set of data, including potency, should be provided at least at the end of the period of shelf life claimed. A possible approach to establishing the shelf life is described in Section 2-2-3 'Stability' General Monograph 0062 of the European Pharmacopoeia 'Vaccines for Veterinary Use' which requires testing until 3 months beyond the claimed shelf-life.
There are no internationally agreed standards or methodology for accelerated stability testing for veterinary vaccines. If manufacturers seek to make claims on the basis of accelerated stability data, validation of the testing methodology and end points should be provided.

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 mimic 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:

- Sterility at time 0 and end of shelf life
- Potency/virus titre/bacterial counts
- 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.
- 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.

> As for 2.G.2. any claims for in-use shelf life of FMD vaccines should be justified following the methodology described below. In view of the lability of the product, manufacturers may recommend using the product 'immediately' on the SPC. Authorities may find this acceptable provided that suitable storage conditions up to the point of use are described.

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/ema/index.jsp?curl=pages/regulation/general/general_content_001639.jsp&mid=WC0b01ac058002ddc6. The guideline places emphasis on conducting the in-use stability study by mimicking the conditions of use of the vaccine in the field.

> The updated link for this guideline is https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-data-requirements-support-use-stability-claims-veterinary-vaccines_en.pdf

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

> The updated link for this guideline to the VICH website is https://vichsec.org/en/index.php?option=com_attachments&view=attachments&task=download&id=152

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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004553.pdf

The reference above provides a link to all of the regulatory guidance for veterinary medicines published on the EMA website

Laboratory testing for safety of FMD vaccines

Laboratory safety studies should be carried out for FMD vaccines in a similar way to any other inactivated viral vaccine. Specific protocols for testing are described below, in the OIE Manual Chapter 3.1.8. Part C. Section 5.2 'Safety' and in VICH GL 44, for which the updated reference is https://www.ema.europa.eu/en/documents/scientific- guideline/vich-gl44-target-animal-safety-veterinary-live-inactived-vaccines-step- 7_en.pdf

Assessors should refer to these guidelines to ensure that the studies provide sufficient assurance of safety. The following specific points should be taken into account for laboratory safety studies of FMD vaccines:

- Batches of vaccine used for safety testing should contain the maximum amount of antigen per strain, and the maximum number of strains, included on the authorisation. This is particularly important in the case of multi-valent FMD vaccines.
- Vaccination of pregnant animals is an important consideration for use of FMD vaccines during control and eradication campaigns. If a claim is made that the vaccine can be administered to pregnant animals, safety

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 immunological veterinary product 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.

> Overdose testing for inactivated vaccines
> Both VICH GL44 (cited in GL2, above) and the European Pharmacopeia Monograph
> 5.2.6. 'Evaluation of safety of veterinary vaccines and immunosera' do not require overdose testing for inactivated vaccines, including FMD vaccines. This test was removed because no additional value could be identified from testing double vs. single doses of inactivated vaccines. GL2 and the OIE FMD Chapter 3.1.8 retain the requirement for overdose testing. National authorities will therefore need to decide whether or not they require overdose testing of FMD vaccines and assessors should follow these local requirements.

3.A.3.Safety of a repeated dose

The immunological veterinary product shall be shown to be safe by considering the number of doses that are likely to be used to vaccinate the animal during its lifetime. 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 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004552.pdf

> The updated link for this guideline is
> https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl41-target- animal-safety-examination-live-veterinary-vaccines-target-animals-absence-reversion_en.pdf

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 immunological veterinary product 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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004598.pdf

> The updated link for this guideline is https://www.ema.europa.eu/en/documents/scientific-guideline/note-guidance-field- trials-veterinary-vaccines_en.pdf

> Field safety testing for FMD vaccines
>
> a) In general, for inactivated vaccines, the results of laboratory safety trials should be supplemented by data from field studies to confirm the safety under the field conditions in line with the OIE Manual Chapter 1.1.8. 'Principles of veterinary vaccine production'. Section 7.2.3. 'Field Tests (safety and efficacy). Field trials present safety data generated in different categories of animals in husbandry conditions representative of those regions.
> b) This GL2 refers applicants to EMEA/CVMP/852/99-Final for further detail on the conduct of field safety trials. This Note for Guidance allows deviations from the basic principle of requiring applicants to conduct field trials '…if there is a zoo- sanitary requirement to restrict the efficacy and safety investigations to laboratory trials.' The epizootic nature of FMD is one such zoo-sanitary requirement.
> c) FMD vaccines may therefore be considered a special case due to the epizootic nature of the disease and the difficulty of carrying out field trials to the standards of Good Clinical Practice defined in VICH GL9 in areas where the disease is endemic. Authorities may decide to accept the absence of field studies provided that comprehensive laboratory safety data is supplied, especially in an emergency situation.
> d) In the absence of field trial data, where available, all additional data generated from the use of the vaccine in the field (publications, reports, pharmacovigilance data etc) should be provided to supplement results of laboratory studies.

3.C. Other Safety issues to be considered

In general, there are no other safety issues that are specific to FMD vaccines and the general guidance below on evaluating environmental risk and ensuring safety to the consumer apply.
FMD vaccine may be formulated with mineral oil adjuvants in the form of a variety of oil emulsions (e.g. water in oil; oil in water; water in oil in water). As for any other inactivated vaccine containing mineral oils, or other reactive adjuvants, applicants should evaluate the safety to users, particularly following self-injection and needle stick injuries. An example of a framework to follow is given in EMA/CVMP/54533/06 'User safety for immunological veterinary medicinal products. Appropriate warnings should be included in the product literature, including advice on the procedure to be followed after self-injection and advice for medical practitioners providing treatment.

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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004574.pdf

The updated link for this guideline is https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-user-safety- immunological-veterinary-medicinal-products_en.pdf

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/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004620.pdf

The updated link for this guideline is https://www.ema.europa.eu/en/documents/scientific-guideline/note-guidance- environmental-risk-assessment-immunological-veterinary-medicinal-products_en.pdf

3.C.3 Safety of residues

Residues studies are not normally required for immunological veterinary products, 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 immunological veterinary product 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/docs/en_GB/document_library/Scientific_guideline/2013/07/WC500146676.pdf

The updated link for this guideline is https://www.ema.europa.eu/en/documents/scientific-guideline/guideline- requirements-combined-vaccines-associations-immunological-veterinary-medicinal- products_en.pdf

PART 4: EFFICACY

In addition to the requirements laid out in this section of GL2, the key requirements for efficacy and potency of FMD vaccines are specified in the OIE Manual Chapter 3.1.8.
Part C. Section 5.3. 'Efficacy' [for registration] Section 5.5. 'Duration of immunity'; and, Section 4.6. 'Potency testing' [for batch release].
As mentioned above under Part 2, it is necessary to cross refer between Part 4: Efficacy and Part 2 Section 2.A. 'Quantitative and Qualitative Particulars' and Section 2.E. 'Control Tests on the Finished Product' to ensure consistency between the information provided in each section as the data generated in the efficacy tests in Part 4 will determine the quantitative particulars in terms of the amount (and types) of antigen to be specified in Section 2.A and the batch control limits to be specified in Section 2.E. In respect to efficacy requirements, FMD vaccines are no different to other inactivated viral vaccines. However, the wide antigenic diversity between strains of the same serotype, the number of species affected and the practical difficulties in carrying out trials under high containment conditions mean that authorities may wish to show a greater degree of flexibility in terms of data requirements whilst maintaining the necessary rigour with respect to standards of safety and efficacy.

For FMD vaccines assessors should note the following:

a) Unusually, the OIE Manual allows efficacy to be demonstrated by challenge with the vaccine strain i.e. the same (homologous) strain as is in the vaccine, provided that it is sufficiently virulent in terms of inducing generalised disease in unvaccinated control animals. Use of the vaccine strain is allowed due to the need to use a challenge strain with a known and clear relationship with the vaccine strain. This test therefore demonstrates the highest possible extent of protection that can be provided by the vaccine. Estimating the likely levels of protection provided by the vaccine against field strains under normal conditions of use is considered below.
b) The definitive tests for demonstrating efficacy of FMD vaccines measure in vivo protection of cattle 21 days after vaccination using a standardised challenge (10^4 Bovine Infectious Doses administered intradermolingually i.e., into the epithelium of the tongue)
c) Protocols are described in Chapter 3.1.8 Section C.5.3 'Efficacy' of the OIE Manual for determining potency (Number of 50% protective doses - PD50; Protection against generalized infection - PGP)
d) Efficacy should be demonstrated by challenge testing at least once for each strain included on an authorization using a monovalent trial blend. Once efficacy has been established and a serological cut-off defined that corresponds to protection, this same cut-off may then be used when the strain is used in combination with any other.
e) In vitro serological tests may subsequently be accepted as evidence of efficacy provided that a correlation has been shown between serological titre and protection and a cut-off defined that corresponds with a vaccine of the minimum potency specified in the authorization. One such test is the Expected Percentage of Protection – EPP described in Section 4.6.1 of Part C of Chapter 3.1.8. of the OIE Manual
f) OIE specifies that vaccines should contain at least 3 PD50 per dose. This corresponds to at least 75% protection in the EPP test. Such vaccines are termed 'Standard' potency in the OIE Manual.
g) There is no internationally agreed potency level for 'Emergency' vaccines. The introductory paragraphs to Part C of the FMD Chapter 3.1.8 indicate that vaccines with a potency ≥6 PD50 can be considered 'higher' potency vaccines and may be suitable for emergency vaccination in naïve populations.

Of tests which have been performed in the target species of animal regarding the efficacy of the IVP 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:

> Efficacy should be demonstrated for each species and category of animal indicated on the label in line with general efficacy requirements for inactivated vaccines. Authorities may wish to show a greater degree of flexibility with respect to demonstrating safety and efficacy in minor species, provided that the requirements have been fully met for at least the major species indicated on the label.

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.

> - Manufacturers should develop and validate challenge models for species other than cattle for these to be included on the label. When claims are made for protection against strains with particular tropism for species other than cattle (e.g. strains from South East Asia that predominantly affect pigs), then efficacy should be fully demonstrated for this species using an appropriate model.
> - The normal requirements for demonstrating the onset and duration of immunity and the effects of maternally derived antibody apply equally to FMD vaccines as to other inactivated viral vaccines. The properties of a vaccine in terms of early onset of immunity, the duration of protection and the minimum age at which animals may be vaccinated are likely to be important parameters in defining an appropriate protocol when vaccination is used to control or eradicate FMD. For these reasons assessors may wish to pay particular attention to the evidence provided to support any claims for these properties in the product literature.
> - Authorities may be prepared to accept serological evidence for duration of immunity once a correlation has been established between antibody level and protection.

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 immunological veterinary product 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.

Comments have been provided under Section 3.B. Field Safety, above, regarding the role of field trials for safety and efficacy in the authorisation of FMD vaccine and these are not repeated here. In summary, authorities may decide to waive the requirements for field trials as part of the authorisation dossier, provided that efficacy has been adequately demonstrated under laboratory conditions and corresponding claims are included in the product literature stating that no data is available for efficacy under field conditions.

Authorities should have in place a national policy on the need for applicants to demonstrate the relevance of vaccine strains in FMD vaccines in order for them to receive a national authorisation. Examples of the national policies that may apply include

- authorities are prepared to authorise vaccines containing any strains of FMD virus provided that the claims reflect the efficacy data shown. This is often the case in countries which are free from FMD and for which any incursion would be considered to be due to an exotic strain against which it would be useful to have authorized vaccines.
- authorities limit authorisation to vaccines that contain strains that have been shown to be relevant for the control of FMD field strains considered to be present within, or represent a risk to, the country. This policy is often applied in countries where the disease is endemic and authorities do not wish to authorise vaccines that are either ineffective or have the potential to interfere with surveillance for FMD in the country. Some countries may restrict the choice of vaccine strains even further to only those that are considered 'local' to avoid importation of strains considered exotic to the country. Such a policy prevents the authorisation of vaccines containing well established vaccine strains that may in fact be more effective in controlling FMD than local strains. Strains differ in their immunogenicity and established vaccine strains have been chosen for their immunogenic potential and ability to grow in culture, properties that may not apply to local strains.
- authorities are prepared to issue marketing authorisations for vaccines containing a wide range of vaccine strains but will prohibit placing on the market of strains that have the potential to interfere with surveillance or eradication. Having a wide range of strains already approved may accelerate the approval for marketing of vaccines containing suitable strains in the event of a change in the epidemiological situation

Assessors should evaluate the information supplied by the applicant in the dossier with respect to choice of vaccine strains and fitness-for-purpose with respect to the legislation and policy that apply to FMD vaccines in their country.

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

Tests that may be applied to evaluate the choice of vaccine strain and the fitness-for- purpose of FMD vaccine are described in the OIE Manual in Chapter 3.1.8;

- Part C Section 1.3 'Validation as a vaccine strain'; describes the requirement for antigenic and genetic characterization of master seed viruses in relation to the original isolate (preferably selected and supplied by a reference laboratory) and to strains circulating in the region in which the vaccine is to be used. This information is usually provided in Part 2 'Quality'.
- Part D 'Vaccine matching Tests'; describes the approach to selecting suitable vaccine and field viruses on which to perform matching tests; methods suitable for measuring the antigenic relationship between vaccine and field strains (usually deriving 'r' values by virus neutralization test (VNT) or ELISA); an approach to correlate serological titres against field viruses with estimated protection as measured by the Estimated Percentage of Protection (EPP) test; and, approaches to testing the fitness-for-purpose of vaccines against field strains involving either challenge or measurement of antibody in vaccinated animals.

Manufacturers may choose to use any appropriate technique to demonstrate the antigenic relationship between vaccine and field strains and assessors should normally expect a combination of antigenic and genetic data. Genetic data alone is rarely sufficient in the case of FMD, as viruses with substantial genetic changes in certain regions can be antigenically related whereas viruses with only a small number of changes in key regions can be antigenically different. Nevertheless, as discussed in Sections 1.3.1 and I.3.2 of the FMD Chapter, genetic information is useful to detect the emergence of new strains and to determine the phylogenetic relationship between strains.

Manufacturers may submit information on vaccine matching studies from international reference laboratories to support the fitness for use of their vaccine strains. The ability to match field strains withvaccine strains relies on access by reference laboratories to the necessary reference sera and vaccine viruses. At the time of preparing these annotations (2021), the OIE/FAO World Reference Laboratory for FMD at the Pirbright Laboratory and the Pan African Veterinary Vaccine Centre of African Union (AU/PANVAC) are working on a project to develop methodology to evaluate the relevance of vaccines for use in Eastern Africa that does not require access to vaccine viruses or reference sera. The approach involves testing post-vaccinal sera by VNT against a panel of FMD viruses of serotypes O, A, SAT1 and SAT 2 that have been chosen to represent the main strains of FMD virus circulating in the region. Information on protocols for testing and the criteria applied for interpreting the results can be found at the following website which will be updated as the project develops:
https://www.wrlfmd.org/fmd-vaccine-quality-control

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

- **Glossary**

Active Immunogenic substance – the active substance in an immunological medicinal product, e.g. a vaccine, which is included as (one of) the antigen(s) of that formulated immunological medicinal product.

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.

Immunological Veterinary Product – a veterinary medicinal product with an immunological mode of action, i.e. it induces immunity to the active substance(s) which it has been formulated.

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.

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

IVP: immunological veterinary product

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 Epizooties (International Office of Epizootics)

rDNA: ribosomal DNA (Deoxyribonucleic acid); it can also mean recombinant DNA which is DNA 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