**USA COMMENTS IN RED FONT**

section 3.9.

suidae

Chapter 3.9.1.

african swine fever
(infection with african swine fever virus)

SUMMARY

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages, caused by ASF virus (ASFV). The clinical syndromes vary from peracute, acute, subacute to chronic, depending on the virulence of the virus. Acute disease is characterised by high fever, haemorrhages in the reticuloendothelial system, and a high mortality rate. Soft ticks of the Ornithodoros genus, especially O. moubata and O. erraticus, have been shown to be both reservoirs and transmission vectors of ASFV. The virus is present in tick salivary glands and passed to new hosts (domestic or wild suids) when feeding. It can be transmitted sexually between ticks, transovarially to the eggs, or transtadially throughout the tick’s life.

ASFV is the only member of the Asfarviridae family, genus Asfivirus.

Laboratory diagnostic procedures for ASF fall into two groups: detection of the virus and serology. The selection of the tests to be carried out depends on the disease situation and laboratory diagnostic capacity in the area or country.

**Identification of the agent:** Laboratory diagnosis must be directed towards isolation of the virus by inoculation of pig leukocyte or bone marrow cultures, the detection of antigen in smears or cryostat sections of tissues by fluorescent antibody test and/or the detection of genomic DNA by the polymerase chain reaction (PCR) or real-time PCR. The PCRs are excellent, highly sensitive, specific and rapid techniques for ASFV detection and are very useful under a wide range of circumstances. They are especially useful if the tissues are unsuitable for virus isolation and antigen detection. In doubtful cases, the material is passaged in leukocyte cell cultures and the procedures described above are repeated.

**Serological tests:** Pigs that survive natural infection usually develop antibodies against ASFV from 7–10 days post-infection and these antibodies persist for long periods of time. Where the disease is endemic, or where a primary outbreak is caused by a strain of low or moderate virulence, the investigation of new outbreaks should include the detection of specific antibodies in serum or extracts of the tissues submitted. A variety of methods such as the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody test (IFAT), the indirect immunoperoxidase test (IPT), and the immunoblotting test (IBT) is available for antibody detection.

**Requirements for vaccines**: ~~At present, there is no vaccine for ASF.~~ Commercially produced modified live virus vaccines are ~~available and licenced~~ under field evaluation in some countries.

A. introduction

The current distribution of African swine fever (ASF) extends across more than 50 countries in three continents (Africa, Asia and Europe). Several incursions of ASF out of Africa were reported between the 1960s and 1970s. In 2007, ASF was introduced into Georgia, from where it spread to neighbouring countries including the Russian Federation. From there ASF spread to eastern European countries extending westwards and reaching the European Union in 2014. Further westward and southern spread in Europe has occurred since that time. In all these countries, both hosts – domestic pig and wild boar – were affected by the disease. In August 2018, the People’s Republic of China reported its first outbreak of ASF and further spread in Asia has occurred. ASF was identified on the island of Hispaniola (Haiti and the Dominican Republic) in 2021. See WAHIS (<https://wahis.woah.org/#/home>) for recent information on distribution at the country level.

ASF virus (ASFV) is a complex large, enveloped DNA virus with icosahedral morphology. It is currently classified as the only member of the *Asfaviridae* family, genus *Asfivirus* (Dixon *et al.*, 2005). More than 60 structural proteins have been identified in intracellular virus particles (200 nm) (Alejo *et al.,* 2018). More than a hundred infection-associated proteins have been identified in infected porcine macrophages, and at least 50 of them react with sera from infected or recovered pigs (Sánchez-Vizcaíno & Arias, 2012). The ASFV double-stranded linear DNA genome comprises between 170 and 193 kilobases (kb) and contains between 150 and 167 open reading frames with a conserved central region of about 125 kb and variable ends. These variable regions encode five multigene families that contribute to the variability of the virus genome. The complete genomes of several ASFV strains have been sequenced (Bishop *et al.,* 2015; Chapman *et al.,* 2011; de Villiers *et al.,* 2010; Portugal *et al.,* 2015). Different strains of ASFV vary in their ability to cause disease, but at present there is only one recognised serotype of the virus detectable by antibody tests.

The molecular epidemiology of the disease is investigated by sequencing of the 3’ terminal end of the B646L open reading frame encoding the p72 protein major capsid protein, which differentiates up to 24 distinct genotypes (Achenbach *et al*., 2017; Boshoff *et al.,* 2007; Quembo *et al.* 2018). To distinguish subgroups among closely related ASFV, sequence analysis of the tandem repeat sequences (TRS), located in the central variable region (CVR) within the B602L gene (Gallardo *et al.,* 2009; Lubisi *et al.,* 2005; Nix *et al.,* 2006) and in the intergenic region between the I73R and I329L genes, at the right end of the genome (Gallardo *et al.,* 2014), is undertaken. Several other gene regions such as the E183L encoding p54 protein, the CP204L encoding p30 protein, and the protein encoded by the EP402R gene (CD2v), have been proved as useful tools to analyse ASFVs from different locations and hence track virus spread.

ASF viruses produce a range of syndromes varying from peracute, acute to chronic disease and subclinical infections. Pigs are the only domestic animal species that is naturally infected by ASFV. European wild boar and feral pigs are also susceptible to the disease, exhibiting clinical signs and mortality rates similar to those observed in domestic pigs. In contrast African wild pigs such as warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*) are resistant to the disease and show few or no clinical signs. These species of wild pig act as reservoir hosts of ASFV in Africa (Costard *et al.,* 2013; Sánchez-Vizcaíno *et al.,* 2015).

The incubation period is usually 4–19 days. The more virulent strains produce peracute or acute haemorrhagic disease characterised by high fever, loss of appetite, haemorrhages in the skin and internal organs, and death in 4–10 days, sometimes even before the first clinical signs are observed. Case fatality rates may be as high as 100%. Less virulent strains produce mild clinical signs – slight fever, reduced appetite and depression – which can be readily confused with many other conditions in pigs and may not lead to suspicion of ASF. Moderately virulent strains are recognised that induce variable disease forms, ranging from acute to subacute. Low virulence, non-haemadsorbing strains can produce subclinical non-haemorrhagic infection and seroconversion, but some animals may develop discrete lesions in the lungs or on the skin in areas over bony protrusions and other areas subject to trauma. Animals that have recovered from either acute, subacute or chronic infections may potentially become persistently infected, acting as virus carriers. The biological basis for the persistence of ASFV is still not well understood, nor it is clear what role persistence plays in the epidemiology of the disease.

ASF cannot be differentiated from classical swine fever (CSF) by either clinical or post-mortem examination, and both diseases should be considered in the differential diagnosis of any acute febrile haemorrhagic syndrome of pigs. Bacterial septicaemias may also be confused with ASF and CSF. Laboratory tests are essential to distinguish between these diseases.

In countries free from ASF but suspecting its presence, the laboratory diagnosis must be directed towards isolation of the virus by the inoculation of pig leukocyte or bone marrow cultures, the detection of genomic DNA by polymerase chain reaction (PCR) or the detection of antigen in smears or cryostat sections of tissues by direct fluorescent antibody test (FAT). Currently the PCR is the most sensitive technique and can detect ASFV DNA from a very early stage of infection in tissues, ethylene diamine tetra-acetic acid (EDTA)-blood and serum samples. The PCR is particularly useful if samples submitted are unsuitable for virus isolation and antigen detection because they have undergone putrefaction. Pigs that have recovered from acute, subacute or chronic infections usually exhibit a viraemia for several weeks making the PCR test a very useful tool for the detection of ASFV DNA in pigs infected with low or moderately virulent strains. Virus isolation by the inoculation of pig leukocyte or bone marrow cultures and identification by haemadsorption tests (HAD) are recommended as a confirmatory test when ASF is positive by other methods, particularly in the event of a primary outbreak or a case of ASF.

~~As no vaccine is available, the presence of ASFV antibodies is indicative of previous infection and, as antibodies are produced from the first week of infection and persist for long periods, they are a good marker for the diagnosis of the disease, particularly in subacute and chronic forms.~~

Vaccines should be prepared in accordance with Chapter 1.1.8 *Principles of veterinary vaccine production*. Current ASF modified live virus (MLVs) vaccines are based on the live virus that have been naturally attenuated or attenuated by targeted genetic recombination through cell cultures (Gladue & Borca, 2022). MLV production is based on a seed-lot system consistent with the *European Pharmacopoeia* (11th edition) and that has been validated with respect to virus identity, sterility, purity, potency~~, stability, safety~~ and immunogenicity ~~(including spread), non-transmissibility, stability and immunogenicity~~. ASF MLV first generation vaccines ~~– defined as those for which peer-reviewed publications are in the public domain –~~ should meet or exceed the minimum standards as described below. ~~Paramount~~ Demonstration of acceptable safety and efficacy against the epidemiologically relevant ASFV field strain(s) circulating in areas where the vaccine is intended for use ~~are~~ is required. At the present time, a variety of mutants (Forth *et al.,* 2023) and recombinants (Zhao *et al.,* 2023) have emerged globally, and the prevalence of these strains is increasing. In addition, there is a risk that vaccine strains will revert to virulence and/or recombine with circulating strains. These conditions should be taken into account ~~in~~ vaccine development. ~~acceptable efficacy should be shown against the B646L (p72) genotype II pandemic virus lineage currently circulating widely in domestic pigs and wild boar.~~

**RATIONALE:** Evidence of genetic instability and reversion to virulence has been observed in recent studies (https://www.federalregister.gov/documents/2022/10/27/2022-23446/notice-of-withdrawal-of-select-agent-regulatory-exclusions-for-two-strains-of-african-swine-fever). Evidence of spread was seen in Tran et al. 2022 "Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-DI177L.

ASF MLV first generation vaccines allowing the differentiation of infected animals from vaccinated animals (DIVA) by suitable methods (e.g. serology-based tests) are preferred. Demonstration of MLV ~~safety and~~ efficacy in pigs at target age and safety at different growth stages (suckling piglets, nursery pigs, fattening pigs), ~~the safety in~~ breeding-age boars, gilts and pregnant sows, ~~and onset and duration of protective immunity,~~ are ~~also preferred but are not~~ required to meet the minimum standard. Additional data will likely be required by Regulatory Authorities if these categories are included in the indications for the vaccine. Details of the onset of immunity (the interval of time elapsed between vaccination and challenge if protection is confirmed) and the duration of immunity (the time point at which vaccine-induced immunity begins to decline and provides less protection) are also required to meet minimum standards.

**RATIONALE:** The efficacy in pigs at different growth stages, breeding-age boars, gilts and pregnant sows can be preferred, but not required. Efficacy is required for the targeted pig groups because the vaccine will be used for the animals in this groups. However, the efficacy for non-targeted groups will be benefit for further improvement of the vaccine, but it is not a required condition because the vaccine does not claim to be used for the animals other than the targeted animals. But the safety in pigs at different growth stages, breeding-age boars, gilts and pregnant sows should be a required condition. Due to the evidence of horizonal spread of MLV, it is essential to demonstrate the safety of a MLV in pigs at different growth stages (suckling piglets, nursery pigs, fattening pigs), in breeding-age boars, gilts and pregnant sows, because horizonal transmission could happen from vaccinated pigs to those non-target pigs.

ASF epidemiology is complex with different epidemiological patterns of infection occurring in Africa and Europe. ASF occurs through transmission cycles involving domestic pigs, wild boar, wild African suids, and soft ticks (Sánchez-Vizcaíno *et al.,* 2015). In regions where *Ornithodoros* ~~soft-bodied~~ ticks are present, the detection of ASFV in these reservoirs of infection contributes to a better understanding of the epidemiology of the disease. This is of major importance in establishing effective control and eradication programmes (Costard *et al*., 2013).

ASF is not a zoonotic disease and does not affect public health (Sánchez-Vizcaíno *et al.*, 2009).

ASFV should be handled with an appropriate level of bio-containment, determined by risk analysis in accordance with Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*.

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c. REQUIREMENTS FOR VACCINES[under review]

~~At present there is no commercially available vaccine for ASF.~~ Commercially produced modified live virus vaccines are being evaluated and licensed for field use .

1. Background

The ASF p72 genotype II strains (ASFV Georgia 2007/1 lineage) (NCBI, 2020) are recognised to be the current highest global threat for domestic pig production worldwide (Penrith *et al.,* 2022). However, genotype I low virulent ~~attenuated~~ strains and genotype I/II recombinant strains have been reported to be circulating. In Africa, multiple genotypes are circulating.

**RATIONALE:** The term "attenuated" better applies to a laboratory procedure that weakens a pathogen. The term "low virulent" is better used when referring to a natural product.

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 *Principles of Veterinary Vaccine Production*. Varying additional requirements relating to quality (including purity and potency), safety, and efficacy will apply in particular countries or regions for manufacturers to comply with local regulatory requirements.

Wherever live, virulent ASFV or ASF MLVs are stored, handled and disposed, the appropriate biosecurity level, procedures and practices should be used. The ASF MLV vaccine production facility should meet the requirements for containment outlined in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*.

An optimal ASF MLV first generation vaccine for the target host should have the following general characteristics (minimum standards):

• Safe: demonstrate absence of persistent fever as defined below (see Section 2.3.2) and clinical signs of acute or chronic ASF in vaccinated and in-contact animals, minimal and ideally no vaccine virus transmission, and absence of an increase in virulence (genetic and phenotypic stability);

• Efficacious: protects against mortality, reduces acute disease (fever accompanied by the appearance of clinical signs caused by ASF) and ~~reduces vertical (boar semen and placental) and~~ reduces vertical (boar semen and placental) andhorizontal disease transmission;

**RATIONALE:** This is an important consideration for field use, especially if vaccine virus is transmitted to wild suids. Certain strains of vaccine virus have been shown to revert to virulence and be transmissible to non-vaccinates. If used in a domestic swine population, vaccine virus could theoretically transmit to wild swine populations, revert to virulence, and cause disease where none was present before. In regard to vertical transmission in wild suids, this could present a long-term problem due only to vaccine virus usage. This is a major concern when considering vaccination before an outbreak vs. vaccination in response to a natural outbreak.

• Quality – purity: free from wild-type ASFV and extraneous microorganisms that could adversely affect the safety, potency or efficacy of the product;

• Quality – ~~potent~~ stability: the ~~log~~~~10~~virus titre maintained throughout the vaccine shelf life that guarantees the efficacy demonstrated by the established minimum immunising (protective) dose.

• ~~Identity~~ Vaccine matching: based on the capacity to protect against the ASFV B646L (p72) genotype II pandemic strain or other p72 genotypes of recognised epidemiologic importance.

Vaccine production should be carried out using a validated, controlled and consistent manufacturing process.

ASF MLV first generation vaccines must be safe (i.e. an acceptable safety profile) for non-target species and the environment in general.

Ideally, ASF MLV first generation vaccines that meet the minimum standards should also fulfil the following additional general characteristics: i) prevents acute and persistent ~~(carrier state)~~ disease; ii) prevents horizontal and vertical disease transmission; iii) induces rapid protective immunity (e.g. < 2 weeks); and iv) confers stable, life-long immunity.

Furthermore, ASF MLV second and future generation vaccines should meet the minimum safety and efficacy standards as ASF MLV first generation vaccines, and ideally provide additional product profile benefits, including but not limited to: i) contain a negative marker allowing the differentiation of infected from vaccinated animals (DIVA) by reliable discriminatory tests such as serology-based tests; and ii) confer broad range of protection against other p72 genotype field strains of varying virulence (low, moderate, and high).

The majority of ASF global vaccine research groups and companies are currently focused on ASF MLV first generation vaccine candidates ~~that are safe and efficacious against~~ ASF viruses belonging to the ASFV p72 genotype II pandemic strain (ASFV Georgia 2007/1 lineage) (NCBI, 2020). More research is needed to determine whether these genotype II-specific MLVs can effectively protect against newly circulating variants of genotype II and recombinant strains.

**RATIONALE:** Certain strains of vaccine virus have been shown to revert to virulence and be transmissible to non-vaccinates. The “current focus” is on a strain that was shown to revert to virulence in certain conditions. The deletion makes this chapter more adaptable as further scientific data is generated/published.

Currently, two recombinant gene deleted MLV ~~recombinant~~ vaccines (ASFV-G-ΔI177L and ASFV-G-ΔMGF) have been licenced ~~for field use~~ in Vietnam for use in domestic pigs ~~following supervised field testing to evaluate the safety and effectiveness of several vaccine batches~~.

There are numerous, promising ASF MLV vaccine candidates targeting the p72 genotype II pandemic strain under development, including:

• A naturally attenuated field strain (Lv17/WB/Rei1) (Barasona *et al.,* 2019) being developed as an oral bait vaccine for wild boar~~s~~;

• A laboratory thermo-attenuated field strain (ASFV-989) (Bourry *et al.,* 2022);

• Single gene-deleted, recombinant viruses (e.g. SY18ΔI226R, ASFV-G-ΔA137R) (Gladue *et al*., 2021; Zhang *et al*., 2021);

• Double gene-deleted, recombinant viruses (e.g. ASFV-G-Δ9GL/ΔUK; ASFV-SY18-∆CD2v/UK; Arm-ΔCD2v-ΔA238L) (O’Donnell *et al*., 2016; Pérez-Núñez *et al*., 2022; Teklue *et al*., 2020);

• Multiple gene-deleted, recombinant viruses (ASFV-G-ΔI177L/ΔLVR; ASFV-G-ΔMGF; ~~BA71ΔCD2;~~ HLJ/18-7GD; ASFVGZΔI177LΔCD2vΔMGF) (Borca *et al*., 2021; Chen *et al*., 2020; Kitamura *et al.,* 2023; Liu *et al*., 2023; Monteagudo *et al*., 2017; O’Donnell *et al*., 2015).

Information regarding many of these MLV vaccine candidates can be found in a recent review publication (Brake, 2022).

Different DIVA strategies using serological methods (e.g. ELISA) or genome detection methods (e.g. differential real-time PCR) are not widely available for these ASF MLV first generation vaccine candidates. Therefore, there is still room for improvement with respect to marker vaccines and their companion diagnostic tests.

Inactivated (non-replicating) whole virus vaccines are not presently available and may be difficult to develop to meet minimum efficacy standards. Recombinant vectored, subunit vaccine candidates that can be produced in scalable vaccine platform expression systems and mRNA-based ASF vaccines are being evaluated in ongoing laboratory research, testing and evaluation in experimental challenge models*.* The publicly available *Center of Excellence for African Swine Fever Genomics* (ASFV Genomics, 2022[[1]](#footnote-2)*)* that provides the structural protein predictions for all 193 ASFV proteins may help accelerate ASF first and second generation vaccine research and development.

Any future use of vaccine candidates should be based on a thorough risk–benefit assessment considering all safety and efficacy features, as well as the potential vaccination scenario. Fit-for-purpose vaccine use scenarios matched to the intended use in a domestic pig-specific type of production system may require different vaccine product profiles or may influence the focus of essential versus ideal vaccine requirements. Prudent use of ASF MLVs as part of strict, controlled vaccination programmes, especially in the areas where ASF is not prevalent, should be implemented. Transmission of vaccine virus to non-vaccinates (domestic or wildlife) is particularly problematic in areas where ASF is not present.

**RATIONALE:** Certain strains of vaccine virus have been shown to revert to virulence and be transmissible to non-vaccinates. If used in a domestic swine population, vaccine virus could theoretically transmit to wild swine populations, revert to virulence, and cause disease where none was present before. This is a major concern when considering vaccination before an outbreak vs. vaccination in response to a natural outbreak.

It is important to know what genotypes of ASFV are circulating in a population before vaccination is introduced. Due to the potential risk of recombination events between circulating low and high virulent field strains with future licensed vaccine strains, and the possibility of reversion to virulence of vaccine strains, strict pharmacovigilance using standard reporting criteria (e.g any fever is reportable – in contrast to the safety testing criteria below of 3 days of fever) post-vaccination is essential. Field pharmacovigilance data should be collected and analysed during vaccination campaigns using ASF MLV first generation vaccines post-licensing. Active post-vaccination surveillance programmes for the detection of new ASF viruses that may arise from MLV vaccine strains and naturally circulating wild-type virus recombination, as well as revertant vaccine strains, should be implemented. It is also recommended that vaccine manufacturers carry out laboratory experiments to further evaluate the risk of vaccine virus reversion to virulence and/or recombination with field and vaccine strains.

**RATIONALE:** Given the looser criteria proposed in other sections of this document for adverse event reporting in licensure studies – e.g. the safety testing criteria – it is worthwhile to note criteria for “strict” pharmacovigilance that is noted as essential to avoid confusion in reporting.

As with any ~~MLV~~ vaccine, all ASF ~~MLV~~ vaccines should be used according to the label instructions, under the strict control of the country’s Regulatory Authority.

The minimum standards given here and in chapter 1.1.8 are intended to be general in nature and may be supplemented by national, regional, and veterinary international medicinal product harmonised requirements. Minimum data requirements for an authorisation in exceptional circumstances (e.g. unexpected introduction of the virus, sudden outbreaks of the disease) should be considered where applicable.

2. Outline of production and minimum requirements for vaccines

2.1. Characteristics of the seed virus

2.1.1. Biological characteristics of the master seed virus

ASF MLVs are generally produced from ASFV field strains derived from naturally attenuated field isolates or using DNA homologous (genetically targeted) recombination techniques in cell cultures to delete one or more ASFV genes or gene families. These molecular techniques typically involve replacement of the targeted ASFV gene(s) with one or more positive, marker fluorescent (e.g. BFP, eGFP, mCherry) or enzyme-based (e.g. β-glucuronidase) ASFV promoter-reporter gene systems that allow the use of imaging microscopy or flow cytometry to visualise, select, and clone gene-deleted, recombinant, ASF MLVs. MLV production is carried out in cell cultures based on a seed-lot system.

Master seed viruses (MSVs) for MLVs should be selected and produced based on their ease of growth in cell culture, virus yield (log10 infectious titre) and genetic stability over multiple cell passages. Preferably, a continuous well-characterised cell line (e.g. ZMAC-4; PIPEC; IPKM) (Borca *et al*., 2021; Masujin *et al*., 2021; Portugal *et al*., 2020) is used to produce a master cell bank (MCB) on which the MSV and MSV-derived working seed virus (WSV) can be produced. The exact source of the underlying ASFV isolate, the whole genome sequence, and the passage history must be recorded.

2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

Only MSVs that have been established as sterile, pure (free of wild-type parental virus and free of extraneous agents as described in Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use*, and those listed by the appropriate licensing authorities) and immunogenic, should be used as the vaccine virus (WSV and vaccine batch production). ~~Live vaccines must be shown not to cause disease or other adverse effects in target animals in accordance with chapter 1.1.8, Section 7.1~~ *~~Safety tests~~* ~~(for live attenuated MSVs), that includes target animal safety tests, increase in virulence tests, assessing the risk to the environment) and if possible, no transmission to other animals.~~

Identity of the MSV must be confirmed using appropriate methods (e.g. through the use of vaccine strain-specific whole genome detection methods such as next generation sequencing).

Demonstration of MSV stability over several cell passages is necessary, typically through at least five passages (e.g. MSV+5). For those MLV vaccines for which attenuation is linked to specific characteristics (gene deletion, gene mutations, etc.), genetic stability of attenuation throughout the production process should be confirmed by full genome sequencing and confirmation of the vaccine phenotype, for example, by confirming the virus titre obtained by growth in the cell line used for production ~~using suitable methods. Suitable techniques to demonstrate genetic stability may include but are not limited to: genome sequencing, biochemical, proteomic, genotypic (e.g. detection of genetic markers) and phenotypic strain characterisation~~. If final product yields (infectious titres) are relatively low, as is typically the case with ASFV, demonstration of stability is required for the maximum passage for use in the final product manufacturing as defined by the producer ~~genetic stability at a minimum of MSV+10 should be demonstrated to allow more flexibility in the outline of production. For example, if MSV+8 is the maximum passage for use in final product manufacturing, demonstration of genetic stability to at least MSV+10 is warranted~~.

2.1.3. Validation as a vaccine strain

The vaccine derived from the MSV must be shown to be satisfactory with respect to safety and efficacy. Live vaccines must be shown not to cause disease or other adverse effects in target animals in accordance with chapter 1.1.8, Section 7.1 *Safety tests* (for live attenuated MSVs), that includes target animal safety tests, increase in virulence tests, assessing the risk to the environment) and if possible, no transmission to other animals.

**RATIONALE:** This text deleted above is appropriate here.

Even if pigs are not known for susceptibility to transmissible spongiform encephalopathy (TSE) agents, consideration should also be given to minimising the risk of TSE transmission by ensuring that animal origin materials from TSE-relevant species, if no alternatives exist for vaccine virus propagation, comply with the measures on minimising the risk of transmission of TSE.

Ideally, the vaccine virus in the final product should generally not differ by more than five passages from the master seed lot.

ASF vaccines should be presented in a suitable pharmaceutical form (e.g. lyophilisate or liquid form).

2.2. Method of manufacture

2.2.1. Procedure

The MLV ~~virus~~ is used to infect swine primary cell cultures obtained from specific-pathogen free pigs, the requirements for which are defined in specific monographs (Chapter 2.3.3 *Minimum requirements for the organisation and management of a vaccine manufacturing facility*, Section 2.4.2). It should be noted that each donor pig should be considered a different “master cell stock” and be tested for purity and extraneous agents to account for the risk of contamination during cell collection and processing. Similar considerations should apply to collections over time at the same time that herd health is closely monitored. Compared with primary cell cultures, use of a continuous cell line generally allows for more consistency, higher serial volumes in manufacturing and aligns better with a seed lot system. Thus, preferably a master cell bank based on an established, continuous cell line shown to support genetically stable ASFV replication and acceptable titres over several passages should be used.

**RATIONALE:** Good to note limitations of primary cell culture production methods.

Cell cultures shall comply with the requirements for cell cultures for production of veterinary vaccines in chapter 1.1.8. Regardless of the production method, the substrate should be harvested under aseptic conditions and may be subjected to appropriate methods to release cell-associated virus (e.g. freeze–thaw cycles, detergent lysis). The harvest can be further processed by filtration and other purification methods. A stabiliser or other excipients may be added as appropriate. The vaccine is homogenised to ensure a uniform batch/serial.

2.2.2. Requirements for ingredients

All ingredients used for vaccine production should be in line with requirements in chapter 1.1.8.

2.2.3. In-process controls

In-process controls will depend on the protocol of production: they include virus titration of bulk antigen and sterility tests.

2.2.4. Final product batch tests

i) Sterility

Tests for sterility and freedom from contamination of biological materials intended for veterinary use may be found in chapter 1.1.9.

ii) Identity

Appropriate methods such as specific genome detection methods (e.g. specific differential real-time PCR) should be used for confirmation of the identity of the vaccine virus and differentiation from the parent strain of the virus as a potential contaminant.

iii) Purity

Appropriate methods should be used to ensure that the final product batch does not contain any residual wild-type ASFV.

iv) Safety

Batch safety testing is to be carried out unless consistent safety of the product is demonstrated and approved in the registration dossier and the production process is approved for consistency in accordance with the standard requirements referred to in chapter 1.1.8.

v) Batch/serial potency

Virus titration is a reliable indicator of vaccine potency once a relationship has been established between the vaccine minimum immunising dose (MID) (minimum protective dose) and titre of the modified live vaccine in vitro. In the absence of a demonstrated correlation between the virus titre and protection, an efficacy test will be necessary (Section C.2.3.3 *Efficacy requirements*, below).

vi) Residual humidity/residual moisture

The test should be carried out consistent with VICH[[2]](#footnote-3) GL26 (*Biologicals: Testing of Residual Moisture*, 2003[[3]](#footnote-4)). Required for MLV vaccines presented as lyophilisates ~~for suspension for injection~~.

2.3. Requirements for authorisation/registration/licensing

2.3.1. Manufacturing process

~~For regulatory approval of a vaccine, a~~All relevant details concerning history of the pre-MSV, preparation of MSV, manufacture of the vaccine and quality control testing (Sections C.2.1 *Characteristics of the seed* and C.2.2 *Method of manufacture*) should be submitted to the authorities.

**RATIONALE:** WOAH chapters should not define acceptance criteria for different regulatory authorities all over the world.

Information shall be provided from three preferably consecutive vaccine batches originating from the same MSV and representative of routine production~~, with a volume not less than 1/10, and more preferably with a volume not less than 1/3 of the typical industrial batch volume~~. The in-process controls are part of the manufacturing process.

**COMMENT:** Suggest reversing the deletion above as this is a good general guideline. If modification is needed, suggest adding "if volumetric standards cannot be met, explain how scale up will be addressed in production".

2.3.2. Safety requirements

For the purpose of gaining regulatory approval, the following safety tests should be performed satisfactorily.

As a minimum standard, vaccines should be tested for any pathogenic effects on healthy domestic pigs of the target age intended for use. Additional demonstration of MLV safety in breeding age gilts and pregnant sows is preferred ~~but not required as a minimum standard~~. If in the future a vaccine intended for use in breeding animals is developed, an evaluation of the impact of the vaccine on reproductive performance will be a standard safety requirement.

i) Safety in young animals

Carry out the test by each recommended route of administration using, in each case, piglets a minimum of ~~6~~ 4-weeks old and not older than 10-weeks old.

The test is conducted using no fewer than eight healthy piglets, and preferably no fewer than ten healthy piglets.

Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine.

Administer to each piglet a quantity of the vaccine virus equivalent to not less than ten times the maximum virus titre (e.g. 50% haemadsorption dose [HAD50], 50% tissue culture infective dose [TCID50]~~, quantitative PCR, etc.~~) (maximum release dose) likely to be contained in one dose of the vaccine.

**COMMENT:** Suggest reversing this deletion as some jurisdictions may choose to use qPCR for potency testing.

To obtain individual and group mean baseline temperatures, the body temperature of each ~~vaccinated~~ piglet is measured on at least the 3 consecutive days preceding administration of the vaccine.

To confirm the presence or absence of fever accompanied by acute and chronic disease, observe the piglets 4 hours after vaccination and then at least once daily for at least 45 days, preferably 60 days post-vaccination. Carry out the daily observations for signs of acute and chronic disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings).

At a minimum of 45 days post-vaccination, humanely euthanise all vaccinated piglets. Conduct gross pathology on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes).

The vaccine complies with the test if:

* No piglet shows abnormal (local or systemic) reactions or notable signs of disease, or reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;
* ~~The average body temperature increase for all vaccinated piglets (group mean) for the observation period does not exceed 1.5°C above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days.~~
* On each day during the observation period, the maximum increase in body temperature above the baseline observed for each pig will be recorded.~~used to calculate the daily group mean temperature rise~~. ~~This mean value should not exceed 1.5°C and n~~No individual pig should show a rise in temperature above baseline greater than 1.5°C for a period exceeding ~~3~~2 consecutive days.
* No vaccinated pigs show notable signs of disease by gross pathology

**RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022.

ii) Safety test in pregnant sows and test for transplacental transmission

There is limited ~~currently an absence of~~ published information on ASFV pathogenesis in breeding-age gilts and in pregnant sows associated with ASFV transplacental infection and fetus abortion/stillbirth. If a label claim is pursued for use in breeding age gilts and sows, then a safety study in line with VICH GL44 (*Guidelines on Target Animal Safety for Veterinary Live and Inactivated Vaccines*, *Section 2.2. Reproductive Safety Test*, 2009[[4]](#footnote-5)) should be completed. The precedent seen with the ASFV-G-Δ9GL single-deletion mutant should be considered when planning and evaluating these studies.

**RATIONALE:** Doses of the single deletion mutant similar to that proposed for the current vaccine strains under consideration (10E4 HAD50) produced a virulent phenotype in inoculated pigs. O’Donnell et al. 2015 doi:10.1128/JVI.00969-15.

iii) Horizontal transmission

The test is conducted using no fewer than 12 healthy piglets, a minimum of ~~6~~ 4-weeks old and not older than 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood samples are negative on real-time PCR. ~~All piglets are housed together from day 0 and the number of vaccinated animals is the same as the number of naïve, contact animals.~~ Co-mingle equal numbers of vaccinated and naïve, contact piglets from day 0 in the same pen or room.

Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. Administer by each recommended route of administration to no fewer than six piglets a quantity of the vaccine virus equivalent to not less than the maximum virus titre (maximum release dose) likely to be contained in 1 dose of the vaccine.

T~~o obtain individual and group mean baseline temperatures, t~~he body temperature of each naïve, contact piglet is measured on at least the 3 consecutive days preceding co-mingling with vaccinated piglets. The body temperature of each naïve, contact piglet is then measured daily for at least 45 days, preferably 60 days.

**RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022.

To confirm the presence or absence of fever accompanied by disease, observe the naïve, contact piglets daily for at least 45 days, preferably 60 days. On each day during the observation period the maximum increase in body temperature above the baseline observed for each pig will be recorded. ~~used to calculate the daily group mean temperature rise. This mean value should not exceed 1.5°C and n~~No individual pig should show a rise in temperature above baseline greater than 1.5°C for a period exceeding 2~~3~~ consecutive days. Carry out the daily observations for signs of acute and chronic clinical disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings.

**RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022.

~~In addition,~~ Blood should be taken from the naïve contact piglets at least twice a week for the first 21 days post-vaccination and then on a weekly basis. From the blood samples, determine vaccine virus titres by quantitative virus isolation (HAD50/ml, TCID50/ml or other methods, e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~infectious virus titres by quantitative virus isolation (e.g. HAD~~~~50~~~~/ml or TCID~~~~50~~~~/ml) and using a real-time PCR test~~.

~~If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only may be used.~~

Collect blood (serum) and oronasal and fecal swabs ~~samples~~ from the naïve contact pigs at least at day 21 and day 28 days and carry out an appropriate test to detect vaccine virus antibodies. At a minimum of 45 days, humanely euthanise all naïve, contact piglets. Conduct gross pathology on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and real-time(RT)-PCR (see Section B.1. Identification of the agent). If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

**RATIONALE:** Relevant sample types to evaluate transmission by all possible routes inserted.

The vaccine complies with the test if:

• No vaccinated or naïve contact piglet shows abnormal (local or systemic) reactions or notable signs of disease, reaches the predetermined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;

• On each day during the observation period the maximum increase in body temperature above the baseline observed for each pig ~~will be used to calculate the daily group mean temperature rise. This mean value should not exceed 1.5°C and no individual pig~~ should show a rise in temperature above baseline greater than 1.5°C for a period exceeding ~~3~~2 consecutive days ~~The average body temperature increase for all naïve, contact piglets (group mean) for the observation period does not exceed 1.5°C: above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days~~;

**RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022

• No naïve, contact piglet shows notable signs of disease by gross pathology ~~and no virus is detected in their blood or tissue samples~~;

* No ~~or a low percentage of~~ contact piglets test both real-time PCR positive and seropositive ~~No naïve contact pigs test positive for antibodies to the vaccine virus~~.

**RATIONALE:** This should not be set to meet pre-determined criteria. The test for horizontal transmission should be reported - whether the data are acceptable to individual regulatory authorities is a separate decision.

iv) Post-vaccination kinetics of viral replication (MLV blood and tissue dissemination) study

Prior to the reversion to virulence study (Section C2.3.2.v. below), a minimum of one study should be performed to determine the post-vaccination kinetics of virus replication in the blood (viremia), tissues and viral shedding.

The test consists of the administration of the vaccine virus from the master seed lot to no fewer than eight healthy piglets, and preferably ten healthy piglets, a minimum of ~~6~~ 4-weeks old and not older than 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood samples are negative on real-time PCR.

Administer to each piglet, using the recommended route of administration most likely to result in spread (such as the intramuscular route or intranasal route), a quantity of the master seed vaccine virus equivalent to not less than the maximum virus titre (maximum release dose) likely to be contained in 1 dose of the final product of the vaccine.

Record daily body temperatures and observe inoculated animals daily for clinical disease for at least 45 days, preferably 60 days.

Carry out the daily observations for signs of acute and chronic clinical disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.* (2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings.

Collect blood samples from all the piglets at least two times per week from 3 days post-vaccination for the first 2 weeks, then weekly for the duration of the test. Determine vaccine virus titres by quantitative virus isolation (HAD50/ml or TCID50/ml) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples but results should be confirmed by infectious virus titration as described above ~~and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only may be used~~.

Determine which blood timepoint(s) should be used in the design of the reversion to virulence study (Section C2.3.2.v. below), for example, specific blood sample(s) at specific timepoints that show the highest titres should be considered for selection and use in the reversion to virulence study.

Collect oral, nasal and faecal swab samples (preferably devoid of blood to minimise assay interference) at least two times per week from 3-days post-vaccination for the first 2 weeks, then weekly for the duration of the test. Test the swabs for the presence of vaccine virus. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/ml or TCID50/ml) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

Euthanise at least two piglets on days 5, 7, 14, 21, and preferably on day 28 (±2 days at each timepoint) and collect spleen, lung, tonsil, kidney tissue samples and at least three different lymph nodes (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes). Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and using real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

Determine which tissue(s) and timepoint(s) should be used to aid in the design of the reversion to virulence study (Section C.2.3.2.v), for example, specific tissues at specific timepoints which show the highest titres should be considered for selection and use in the reversion to virulence study.

v) Reversion to virulence

The test carried out should be consistent with VICH GL41 (Examination of live veterinary vaccines in target animals for absence of reversion to virulence, 2008[[5]](#footnote-6)).

The test for increase in virulence consists of the administration of the vaccine master seed virus to healthy piglets of an age (e.g. between ~~6~~ 4 weeks and 10 weeks old) suitable for recovery of the strain and of the same origin, that do not have antibodies against ASFV, and blood samples that are negative on real-time PCR. This protocol is typically repeated five times.

*First passage* (*p1*)

Administer to no fewer than two piglets, and preferably no fewer than four piglets using the intended route of administration for the final product, a quantity of the master seed vaccine virus equivalent to not less than the maximum virus titre (maximum release dose) likely to be contained in 1 dose of the master seed of the ~~final product of the~~ vaccine. Observe inoculated animals daily for the appearance of ~~at least two and preferably at least three~~ clinical signs ~~and record daily body temperatures~~ using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a) and record daily body temperatures.

**RATIONALE:** The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) defines worldwide standards for vetrinary biologics. Guideline 41 specified master seed for this study design as passages of the master seed may be more attenuated, and this evaluation should be on the virus most likely to revert to virulence. <https://www.vichsec.org/en/index.php?option=com_attachments&view=attachments&task=download&id=321>

Based on results from at least one completed post-vaccination kinetics of viral replication (MLV ~~vaccine shed and spread (virus~~ blood and tissue dissemination study (Section C.2.3.2.iv above), collect an appropriate quantity of blood from each piglet on the predetermined ~~single~~ timepoint(s) (day ~~5~~ 3–13). Determine virus titres in individual bloodsamples by quantitative virus isolation (HAD50/ml or TCID50/ml) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and by real-time PCR. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~. Identify the individual blood sample(s) with the highest infectious titre and reserve for the subsequent *in-vivo* passage (second pass, p2).

Based on results from at least one completed ~~vaccine virus~~ MLV blood and tissue ~~distribution~~ dissemination study (Section C.2.3.2.iv above), euthanise piglets on the predetermined timepoint (i.e. day 5, 7, 14, 21, or 28). Determine infectious virus titres in individual tissue samples by quantitative virus isolation (HAD50/ml or TCID50/ml) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above. ~~If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used.~~ Identify individual tissuesample type(s) with the highest infectious titre. Pool the tissues with the highest titres from different organs from ~~all~~ each animal~~s~~ ~~with the highest titres~~ and prepare ~~at least~~ a ~~10%~~ virus suspension ~~to obtain a virus titre within the range used for inoculation~~ in PBS, pH 7.2 kept at 4°C or at –70°C for longer storage.

**RATIONALE:** The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) defines worldwide standards for vetrinary biologics. Guideline 41 specifies “The initial administration and subsequent passages shall be carried out using a recommended route of administration or natural route of infection that is the most likely to lead to reversion to or increase in virulence and result in recovery of the organism following replication in the animal.” Diluting the virus to the range used in the initial inoculum instead of passing all recovered virus could decrease the likelihood of detecting a reversion to virulence.

Test each blood and tissue sample pool used for inoculation by PCR to confirm the absence of potential viral agent contaminants (i.e. CSFV, FMDV, PRRS, PCV2). Blood and pooled tissue (p1) are used to inoculate 2 ml of positive material ~~diluted to the maximum release dose likely to be contained in 1 dose of the vaccine~~ using the intended route of administration for the final product to each of at least two and ideally at least four further pigs of the same age and origin.

**RATIONALE:** These should not be diluted or manipulated to increase the chance of detecting reversion to virulence.

*~~Second pass~~* ~~(~~*~~p2~~*~~)~~

If no virus is found at passage 1 (p1), repeat the administration by the intended route ~~once~~ again with the same pooled material (blood and pooled tissue, p1) in another ten healthy piglets of the same age and origin. If no virus is found at this point ~~during this second passage (p2) at this point~~, end the process here.

*Second passage* (*p2*)

If ~~however~~ virus is found in p1, carry out a second series of passages by administering 2 ml of positive material ~~diluted to the maximum release dose likely to be contained in 1 dose of the vaccine~~ using the intended route of administration for the final product to each of no fewer than two piglets, and preferably no fewer than four piglets of the same age and origin. Observe inoculated animals daily for the appearance of ~~at least two and preferably at least three~~ clinical signs using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a), ~~and~~ record daily body temperatures and determine infectious virus titres in individual blood and tissue samples as described for p1 above.

**RATIONALE:** These should not be diluted or manipulated to increase the chance of detecting reversion to virulence.

*~~Third and fourth pass~~* ~~(~~*~~p3 and p4~~*~~)~~

If no virus is found at ~~in~~ ~~(~~p2~~)~~, repeat the ~~intramuscular~~ administration by the intended route ~~once again~~ with the same pooled material (blood and pooled tissue, p2) in another eight healthy piglets of the same age and origin. If no virus is found at this point, end the process here.

*Third and fourth passage* (*p3 and p4*)

If~~, however,~~ virus is found on p2, carry out this passage operation no fewer than two additional times (p3 and p4) (to each of no fewer than two piglets, and preferably no fewer than four piglets of the same age and origin) and verifying the presence of the virus at each passage in blood and tissues. Observe inoculated animals daily for the appearance of ~~at least two and preferably at least three~~ clinical signs using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a) and record daily body temperatures.

*Fifth passage* (*p5*)

Administer 2 ml of the blood and pooled tissue (p4) to each of at least eight healthy piglets of the same age and origin. Observe inoculated animals daily for at least 28 days post-inoculation for the appearance of ~~at least two and preferably at least three~~ clinical signs using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a), ~~and~~ record daily body temperature and determine infectious virus titres in individual blood and tissue samples as described above.

The vaccine virus complies with the test if:

• No piglet shows abnormal (local or systemic) reactions, ~~or notable signs of disease, or~~ reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine; and

**COMMENT:** Suggest reversing deletion above given recent data on U.S. select agent exclusion recission. We don’t know what clinical signs will be caused by revertant vaccine strains – this consideration should be broad and inclusive (local or systemic reactions and/or notable signs of disease).

• There is no indication of increasing virulence (as monitored by daily body temperature accompanied by clinical sign observations) of the maximally passaged virus compared with the master seed virus.

At a minimum, a safe MLV vaccine shall demonstrate ALL the following features (minimal standards):

* Absence of fever (on each day during the observation period, the maximum increase in body temperature above the baseline observed for each pig will be recorded, ~~will be used to calculate the daily group mean temperature rise. This mean value should not exceed 1.5°C~~ and no individual pig should show a rise in temperature above baseline greater than 1.5°C ~~(defined as average body temperature increase for all vaccinated piglets (group mean) for the observation period does not exceed 1.5°C above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C~~ for a period exceeding 2 ~~3~~ days);
* **RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022.
* Absence of chronic and acute clinical signs and gross pathology over the entire test period or ~~minimal chronic~~ mild clinical signs (~~defined as~~ e.g. mild swollen joints with a low clinical score that resolve within 1 week).
* Minimal (defined as no naïve, contact piglet shows notable signs of disease by clinical signs and gross pathology and no or a low percentage of contact piglets test both real-time PCR positive and seropositive) or no vaccine virus transmission (defined as no naïve, contact piglet shows notable signs of disease by clinical signs and gross pathology and no contact piglets test both real-time PCR positive and seropositive) over the entire test period;
* Absence of an increase in virulence (genetic and phenotypic stability) (complies with the reversion to virulence test).

In addition, for regulatory approval, ASF MLV ~~the~~ vaccines ~~in their commercial presentation before being authorised for general use~~ should be tested for safety ~~in the~~ under field conditions (see chapter 1.1.8 Section 7.2.3). ~~Additional~~ Field safety studies generally ~~evaluation studies~~ may include measurement of body temperatures, observation of local or systemic reactions and, where appropriate, performance measurements such as: environmental persistence (e.g. determination of virus recovery from bedding or other surfaces), assessment of immunosuppression, and negative impacts on performance. ~~but are not limited to: environmental persistence (e.g. determination of virus recovery from bedding or other surfaces), assessment of immunosuppression, and negative impacts on performance~~.

**COMMENT:** Suggest reversing deletion above. Environmental persistence is a very relevant criterion for evaluation given recent data on U.S. select agent exclusion recission and the potential for reversion to virulence and spread to wild suids. Other criteria are also valid given the unknown clinical symptoms that might be seen with revertant viruses and are listed as examples for incoporation.

2.3.3. Efficacy requirements

i) Protective dose

Vaccine efficacy is estimated in immunised animals directly, by evaluating their resistance to live virus challenge. The test consists of a vaccination/challenge trial in piglets a minimum of ~~6~~ 4-weeks old and not more than 10-weeks old, free of antibodies to ASFV, and negative blood samples by real-time PCR. The test is conducted using no fewer than 15 and preferably no fewer than 24 vaccinated pigs, and no fewer than five non-vaccinated control piglets.

The test is conducted to determine the minimal immunising dose (MID) (also referred to as the minimal protective dose [MPD] or protective fraction); using at least three groups of no fewer than five and preferably not fewer than eight vaccinated piglets per group, and one additional group of no fewer than five non-vaccinated piglets of the same age and origin as controls. Use vaccine containing virus at the highest passage level that will be present in a batch of vaccine.

Each group of piglets, except the control group, is immunised with a different vaccine virus content in the same vaccine volume. In at least one vaccinated group, piglets are immunised with a vaccine dose containing not more than the minimum virus titre (minimum release dose) likely to be contained in one dose of the vaccine as stated on the label.

A suitable challenge model should be developed based on anticipated field usage of the vaccine. As a baseline protocol, ~~T~~twenty-eight days (±2 days) after the single ~~injection~~ dose of vaccine (or if using two ~~injections~~ doses of the vaccine then 28 days [±2 days] following the second ~~injection~~ dose), challenge all the piglets by the intramuscular route. If previous studies have demonstrated acceptable efficacy using IM challenge, then a different challenge route (e.g. direct contact, oral or oronasal) may be used. Challenged, vaccinated piglets may be housed in one or more separate pens in the same room or in different rooms. Challenged, naïve controls can be housed in one or more rooms that are separate from challenged, vaccinated piglets.

**RATIONALE:** National Veterinary Joint Stock Company (NAVETCO) updated label claims to allow a single vaccine dose. Suggest this modification to allow different regulatory authorities flexibility to pursue their own criteria for protection.

Carry out the test using an ASFV representative strain of the epidemiologically relevant field strain(s) where the vaccine is intended for use (e.g. ASFV B646L [p72] genotype II pandemic strain and other p72 virulent genotype of recognised epidemiologic importance). For gene deleted, recombinant MLV viruses, if neither challenge virus type is available, then carry out the test with the parental, virulent virus used to generate the MLV recombinant virus. Use a 10e3–10e4 HAD50 (or TCID50 for non-HAD viruses) challenge dose sufficient to cause death or meet the humane endpoint in 100% of the nonvaccinated piglets in less than 21 days. Higher or lower challenge doses can be considered if appropriately justified.

The rectal temperature of each vaccinated piglet is measured on at least the 3 days preceding administration of the challenge virus, at the time of challenge, 4 hours after challenge, and then daily for the observation period of at least ~~28~~ 45 days, preferably ~~35~~ 60 days. ~~Observe the piglets at least daily for at least 28 days, preferably 35 days.~~ Carry out the daily observations for signs of acute and chronic clinical disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.*, 2015). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings.

Collect oral, nasal, and fecal ~~anal~~ swabs, and blood samples from the vaccinated challenged piglets at least two times ~~once~~ per week from 3 days post-challenge for at least ~~28~~ 14 days, then weekly up to 35 days post-challenge and then every 14 days up to the end of the observation period ~~preferably 35 days~~. From the blood samples, determine infectious virus titres by quantitative virus isolation (HAD50/ml or TCID50/ml) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only may be used~~.

**RATIONALE:** Relevant sample types to evaluate transmission by all possible routes inserted.

At the end of the test period, humanely euthanise all vaccinated challenged piglets. Conduct gross pathology (and histopathology if considered necessary) on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes). Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~and real-time PCR (see Section B.1. Identification of the agent). If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

The test is invalid if ~~fewer than 100%~~ the difference between ~~in~~ the number of unvaccinated control piglets infected with the live challenge virus and the number of vaccinated / challenged piglets ~~vaccinated with the minimum release dose~~ that die or reach a humane endpoint is not statistically significant.

The vaccine (or a specific vaccine virus dose if conducting a vaccine dose titration study) complies with the test if:

• No vaccinated challenged piglet dies or ~~shows abnormal (local or systemic) reactions,~~ reaches the humane endpoint ~~or dies~~ from causes attributable to ASF;

**COMMENT:** Suggest reversing deletion above given recent data on U.S. select agent exclusion recission. We don’t know what clinical signs will be caused by revertant vaccine strains – this consideration should be broad and inclusive (local or systemic reactions and/or notable signs of disease).

• On each day during the observation period the maximum increase in body temperature above the baseline observed for each pig will be recorded. ~~used to calculate the daily group mean. This mean value should not exceed 1.5°C and~~ nNo individual pig should show a rise in temperature above baseline greater than 1.5 ~~2.0~~°C for a period exceeding 2 consecutive days T~~he average body temperature increase for all vaccinated challenged piglets (group mean) for the observation period does not exceed 2.0°C above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.0°C~~;

**RATIONALE:** These criteria are set to be acceptable for the current, most developed vaccine strain and are not objectively developed. Additionally, taking the average temperature of the group could mask individual reactions. Minimal temperature rise of 1 day was seen in safety studies - 3 days seems long based on current data. Tran et al. 2022.

• The vaccinated challenged piglets display a reduction or absence of typical acute clinical signs of disease and gross pathology and a reduction or absence of challenge virus levels in blood and tissues.

ii) Assessment for horizontal transmission (challenge virus shed and spread study)

~~The ASF basic reproduction number, R0, can be defined as the average number of secondary ASF disease cases caused by a single ASFV infectious pig during its entire infectious period in a fully susceptible population (Hayes~~ *~~et al~~*~~., 2021). In general, if the ASFV effective reproduction number Re=R0 × (S/N) (S= susceptible pigs; N= total number of pigs in a given population) is greater than 1.0, disease is predicted to spread. Ideally, ASF vaccination should reduce Re to less than 1.0 by reducing the number of susceptible, naïve, contact pigs exposed to vaccinated, infected pigs.~~

**RATIONALE:** These are population level statistics and are not valid when calculated with small sample sizes in defined settings.

To evaluate ASF vaccine impact on ASF disease transmission, the test consists of a vaccination/challenge trial in piglets a minimum of ~~6~~ 4-weeks old and not older than 10-weeks old, free of antibodies to ASFV, and negative blood samples by real-time PCR.

The test is conducted using no fewer than 15 healthy piglets at a ratio comprising twice the number of vaccinated piglets to naïve piglets (e.g. ten vaccinated and five naïve). Use vaccine containing virus at the highest passage level that will be present in a batch of the vaccine.

The quantity of vaccine virus administered to each pig is equivalent to be not less than the minimum virus titre (minimum dose) likely to be contained in one dose of the vaccine as stated on the label. Following immunisation, vaccinated and naïve piglets should continue to be co-mingled.

Twenty-eight days (±2 days) after the single ~~injection~~ dose of vaccine (or if using two ~~injections~~ doses of the vaccine then 28 days [±2 days] following the second ~~injection~~ dose), temporarily separate [into different pen(s) or room(s)] all vaccinated piglets from naïve piglets. Challenge all vaccinated piglets by the intramuscular or other previously verified route. Carry out the challenge using an ASFV representative strain of the epidemiologically relevant field strain(s) where the vaccine is intended for use (e.g. ASFV B646L [p72] genotype II pandemic strain and other p72 virulent genotype of recognised epidemiological importance). For gene deleted, recombinant MLV viruses, if neither challenge virus type is available, then carry out the test with the parental, virulent virus used to generate the MLV recombinant virus. Use a 10e3–10e4 HAD50 (or TCID50 for non-HAD viruses challenge dose sufficient to cause death or met the humane endpoint in 100% of the nonvaccinated piglets in less than 21 days. Higher or lower challenge doses can be considered if appropriately justified.

Approximately 18–24 hours later, re-introduce naïve piglets to vaccinated, challenged piglets and allow for direct nose to nose contact exposure with vaccinated, challenged piglets. Allow for continuous contact exposure by co-mingling both groups through the end of the study. If more than one pen or room is used for co-housing, following reintroduction initially maintain a ratio of 2:1 of challenged, vaccinated piglets to contact exposed, naïve piglets.

The rectal temperature of each contact piglet is measured on at least the 3 days preceding administration of the challenge virus to vaccinated pigs, immediately prior to direct contact exposure, 4 hours post-contact exposure, and then daily for at least 28~~, preferably 35~~ days and twice a week for at least 60 days. Observe all contact exposed piglets ~~at least~~ daily for at least 28 days, and then twice a week for at least 60 days ~~preferably for at least 35 days~~.

Carry out the ~~daily~~ observations in each contact piglet for signs of acute and chronic clinical disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,* 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings.

In addition, ~~blood should be taken from the naïve contact piglets at least twice a week from 3 days post-contact exposure for the duration~~ collect blood samples from the contact piglets at least two times per week from 3 days post-contact for at least 14 days, then weekly up to 35 days post-contact exposure and then every 14 days up to the end of the test period. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~From the blood samples, determine infectious challenge virus titres by quantitative virus isolation (HAD~~~~50~~~~/ml or TCID~~~~50~~~~/ml) and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only may be used~~.

Collect blood (serum) samples from the naïve contact pigs at least at day 21 and day 28 (±2 days), and at the end of the test period, and carry out an appropriate test to detect vaccine virus antibodies.

Collect oral, nasal and faecal swab samples (preferably devoid of blood to minimise assay interference) from all contact-exposed naïve piglets at least two times per week from 3-days post-contact exposure for the first 2 weeks, then weekly for the duration of the test and test swabs for the presence of challenge virus. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~Determine virus titres in all collected samples by quantitative virus isolation (HAD~~~~50~~~~/ml or TCID~~~~50~~~~/ml) and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

At the end of the test period, humanely euthanise all contact piglets. Conduct gross pathology on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes. (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes). Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg) or other appropriate methods (e.g. titration using IPT or FAT detection). Quantitative PCR may be used to detect positive samples, but results should be confirmed by infectious virus titration as described above ~~Determine virus titres in all collected samples by quantitative virus isolation (HAD~~~~50~~~~/mg or TCID~~~~50~~~~/mg) and real-time PCR (see Section B.1. Identification of the agent). If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection) may be used~~.

The test is invalid if the vaccine fails to comply with the compliance criteria described for the protected dose test in vaccinated pigs (Section C.2.3.3.i above) or evidence of disease is seen in non-vaccinated pigs.

**RATIONALE:** This section is evaluation of horizontal transmission and did not include evidence of disease in non-vaccinates as a criteria as the reference is to an efficacy evaluation.

If the manufacturer claims that the vaccine induces sterilising immunity, the vaccine complies with the test for a reduction in horizontal disease transmission if all the following conditions are satisfied:

• No naïve, contact exposed piglet shows abnormal (local or systemic) reactions, reaches the defined humane endpoint or dies from causes attributable to ASF;

• No naïve, contact exposed piglet displays fever accompanied by typical signs of disease, including gross pathology.

• Naïve contact pigs show an absence of challenge virus in blood and tissues.

• No naïve contact pigs test positive for antibodies to the challenge virus.

Otherwise, the vaccine complies with the test for a reduction in horizontal disease transmission if:

* Naïve contact pigs show a reduction or absence of challenge virus levels in blood and tissues.
* None of or a reduced number of naïve contact exposed pigs test positive for antibodies to the challenge virus.

At a minimum, an efficacious MLV vaccine shall demonstrate ALL the following features (minimal standards):

• Protects against mortality;

• Reduces acute disease (fever accompanied by a reduction of typical clinical and pathological signs of acute disease)

• Reduces levels of viral shedding and viraemia.

• Reduces horizontal disease transmission (~~no~~ none of or a reduced number of naïve, contact exposed piglets shows abnormal [local or systemic] reactions, reaches the humane endpoint or dies from causes attributable to ASF, ~~and~~ displays fever accompanied by typical acute disease signs caused by ASF), test positive for antibodies to the challenge virus.

* ~~Reduces levels of viral shedding and viraemia.~~

In general, for regulatory approval, ASF MLV ~~addition, the~~ vaccines ~~in their commercial presentation before being authorised for general use~~ should be tested for efficacy ~~in the~~ under field conditions (see chapter 1.1.8 Section 7.2.3). ~~Additional~~ Field efficacy ~~evaluation~~ studies ~~may~~ generally include ~~but are not limited to: onset of immunity, duration of immunity, and impact on disease transmission~~ measurement of relevant efficacy parameters including but limited to mortality, clinical signs, impact on disease transmission, performance parameters.

2.3.4. Duration of immunity

Although not included in the guidance for ASF MLV first generation vaccines, manufacturers are ~~encouraged~~ required, as part of the authorisation procedure, to define and demonstrate the duration of immunity of a given vaccine ~~by evaluation of potency at the end of the claimed period of protection~~.

2.3.5. Stability

Stability of the vaccine should be demonstrated over the shelf life recommended for the product. Although not included in the standards for first generation MLV ASF vaccines, manufacturers are ~~encouraged~~ required, as part of the authorisation procedure, to generate data supporting the retention of immunogenicity over a defined period of ~~validity~~ time of a lyophilised or other pharmaceutical form of the ASF vaccine as part of the authorisation procedure.

**RATIONALE:** Suggest reversal of all these changes - this should not be required for an initial approval.

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**NB:** There are WOAH Reference Laboratories for African swine fever
(please consult the WOAH Web site:
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).
Please contact the WOAH Reference Laboratories for any further information on
diagnostic tests and reagents for African swine fever

**NB:** First adopted in 1990. Most recent updates adopted in 2021.

1. http://asfvgenomics.com. Accessed 4/4/2023. [↑](#footnote-ref-2)
2. VICH: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products [↑](#footnote-ref-3)
3. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl26-biologicals-testing-residual-moisture-step-7\_en.pdf [↑](#footnote-ref-4)
4. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl44-target-animal-safety-veterinary-live-inactived-vaccines-step-7\_en.pdf. [↑](#footnote-ref-5)
5. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl41-target-animal-safety-examination-live-veterinary-vaccines-target-animals-absence-reversion\_en.pdf. [↑](#footnote-ref-6)