* 1. **Annex 16. Item 5.1. – Chapter 3.9.1. African swine fever (infection with African swine fever virus)**
  2. MEETING OF THE BIOLOGICAL STANDARDS COMMISSION
  3. **Paris, 4–8 September 2023**

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5 **SEC T I O N 3. 9.**

# 6 S U I D A E

**USA COMMENTS IN RED FONT**

7 **CHAP T E R 3 . 9 . 1 .**

# 8 A F R I C A N S WI N E F EV E R

9 **( I N F E C T I ON WI T H A F R I CA N SWI N E FEVER VI R US)**

10 **SUMMARY**

1. *African swine fever* (*ASF*) *is an infectious disease of domestic and wild pigs of all breeds and ages, caused*
2. *by ASF virus* (*ASFV*)*. The clinical syndromes vary from peracute, acute, subacute to chronic, depending on*
3. *the virulence of the virus. Acute disease is characterised by high fever, haemorrhages in the*
4. *reticuloendothelial system, and a high mortality rate. Soft ticks of the* Ornithodoros *genus, especially* O.
5. moubata *and* O. erraticus*, have been shown to be both reservoirs and transmission vectors of ASFV. The*
6. *virus is present in tick salivary glands and passed to new hosts* (*domestic or wild suids*) *when feeding. It can*
7. *be transmitted sexually between ticks, transovarially to the eggs, or transtadially throughout the tick’s life. In an outbreak, animal-to-animal transmission (direct contact with infected pigs or their fluids) is an important method of spread.*
8. **RATIONALE:** In a study under field conditions, 50% of the pigs in the comingled contact group developed ASFV-specific antibodies. This appears to confirm a certain level of contact transmission. <https://www.mdpi.com/1999-4915/14/5/896>.
9. *ASFV is the only member of the* Asfarviridae *family, genus* Asfivirus*.*
10. *Laboratory diagnostic procedures for ASF fall into two groups: detection of the virus and serology. The*
11. *selection of the tests to be carried out depends on the disease situation and laboratory diagnostic capacity*
12. *in the area or country.*
13. ***Identification of the agent:*** *Laboratory diagnosis must be directed towards isolation of the virus by*
14. *inoculation of pig leukocyte or bone marrow cultures, the detection of antigen in smears or cryostat sections*
15. *of tissues by fluorescent antibody test and/or the detection of genomic DNA by the polymerase chain reaction*
16. (*PCR*) *or real-time PCR. The PCRs are excellent, highly sensitive, specific and rapid techniques for ASFV*
17. *detection and are very useful under a wide range of circumstances. They are especially useful if the tissues*
18. *are unsuitable for virus isolation and antigen detection. In doubtful cases, the material is passaged in*
19. *leukocyte cell cultures and the procedures described above are repeated.*
20. ***Serological tests:*** *Pigs that survive natural infection usually develop antibodies against ASFV from 7–10*
21. *days post-infection and these antibodies persist for long periods of time. Where the disease is endemic, or*
22. *where a primary outbreak is caused by a strain of low or moderate virulence, the investigation of new*
23. *outbreaks should include the detection of specific antibodies in serum or extracts of the tissues submitted.*
24. *A variety of methods such as the enzyme-linked immunosorbent assay* (*ELISA*)*, the indirect fluorescent*
25. *antibody test* (*IFAT*)*, the indirect immunoperoxidase test* (*IPT*), *and the immunoblotting test* (*IBT*) *is available*
26. *for antibody detection.*
27. ***Requirements for vaccines****: ~~At present, there is no vaccine for ASF.~~ Commercially produced modified live*
28. *virus vaccines are available and licenced in some countries.*

## 38 A. INTRODUCTION

1. The current distribution of African swine fever (ASF) extends across more than 50 countries in three continents (Africa,
2. Asia and Europe). Several incursions of ASF out of Africa were reported between the 1960s and 1970s. In 2007, ASF was
3. introduced into Georgia, from where it spread to neighbouring countries including the Russian Federation. From there ASF
4. spread to eastern European countries extending westwards and reaching the European Union in 2014. Further westward
5. and southern spread in Europe has occurred since that time. In all these countries, both hosts – domestic pig and wild
6. boar – were affected by the disease. In August 2018, the People’s Republic of China reported its first outbreak of ASF and
7. further spread in Asia has occurred.
8. ASF virus (ASFV) is a complex large, enveloped DNA virus with icosahedral morphology. It is currently classified as the
9. only member of the *Asfaviridae* family, genus *Asfivirus* (Dixon *et al.*, 2005). More than 60 structural proteins have been
10. identified in intracellular virus particles (200 nm) (Alejo *et al.,* 2018). More than a hundred infection-associated proteins
11. have been identified in infected porcine macrophages, and at least 50 of them react with sera from infected or recovered
12. pigs (Sánchez-Vizcaíno & Arias, 2012). The ASFV double-stranded linear DNA genome comprises between 170 and
13. 193 kilobases (kb) and contains between 150 and 167 open reading frames with a conserved central region of about 125
14. kb and variable ends. These variable regions encode five multigene families that contribute to the variability of the virus
15. genome. The complete genomes of several ASFV strains have been sequenced (Bishop *et al.,* 2015; Chapman *et al.,*
16. 2011; de Villiers *et al.,* 2010; Portugal *et al.,* 2015). Different strains of ASFV vary in their ability to cause disease, but at
17. present there is only one recognised serotype of the virus detectable by antibody tests.
18. The molecular epidemiology of the disease is investigated by sequencing of the 3’ terminal end of the B646L open reading
19. frame encoding the p72 protein major capsid protein, which differentiates up to 24 distinct genotypes (Achenbach *et al*.,
20. 2017; Boshoff *et al.,* 2007; Quembo *et al.* 2018). To distinguish subgroups among closely related ASFV, sequence analysis
21. of the tandem repeat sequences (TRS), located in the central variable region (CVR) within the B602L gene (Gallardo *et*
22. *al.,* 2009; Lubisi *et al.,* 2005; Nix *et al.,* 2006) and in the intergenic region between the I73R and I329L genes, at the right
23. end of the genome (Gallardo *et al.,* 2014), is undertaken. Several other gene regions such as the E183L encoding p54
24. protein, the CP204L encoding p30 protein, and the protein encoded by the EP402R gene (CD2v), have been proved as
25. useful tools to analyse ASFVs from different locations and hence track virus spread.
26. ASF viruses produce a range of syndromes varying from peracute, acute to chronic disease and subclinical infections.
27. Pigs are the only domestic animal species that is naturally infected by ASFV. European wild boar and feral pigs are also
28. susceptible to the disease, exhibiting clinical signs and mortality rates similar to those observed in domestic pigs. In contrast
29. African wild pigs such as warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus porcus*) and giant forest hogs
30. (*Hylochoerus meinertzhageni*) are resistant to the disease and show few or no clinical signs. These species of wild pig act
31. as reservoir hosts of ASFV in Africa (Costard *et al.,* 2013; Sánchez-Vizcaíno *et al.,* 2015).
32. The incubation period is usually 4–19 days. The more virulent strains produce peracute or acute haemorrhagic disease
33. characterised by high fever, loss of appetite, haemorrhages in the skin and internal organs, and death in 4–10 days,
34. sometimes even before the first clinical signs are observed. Case fatality rates may be as high as 100%. Less virulent
35. strains produce mild clinical signs – slight fever, reduced appetite and depression – which can be readily confused with
36. many other conditions in pigs and may not lead to suspicion of ASF. Moderately virulent strains are recognised that induce
37. variable disease forms, ranging from acute to subacute. Low virulence, non-haemadsorbing strains can produce subclinical
38. non-haemorrhagic infection and seroconversion, but some animals may develop discrete lesions in the lungs or on the
39. skin in areas over bony protrusions and other areas subject to trauma. Animals that have recovered from either acute,
40. subacute or chronic infections may potentially become persistently infected, acting as virus carriers. The biological basis
41. for the persistence of ASFV is still not well understood, nor it is clear what role persistence plays in the epidemiology of
42. the disease.
43. ASF cannot be differentiated from classical swine fever (CSF) by either clinical or post-mortem examination, and both
44. diseases should be considered in the differential diagnosis of any acute febrile haemorrhagic syndrome of pigs. Bacterial
45. septicaemias may also be confused with ASF and CSF. Laboratory tests are essential to distinguish between these
46. diseases.
47. In countries free from ASF but suspecting its presence, the laboratory diagnosis must be directed towards isolation of the
48. virus by the inoculation of pig leukocyte or bone marrow cultures, the detection of genomic DNA by polymerase chain
49. reaction (PCR) or the detection of antigen in smears or cryostat sections of tissues by direct fluorescent antibody test
50. (FAT). Currently the PCR is the most sensitive technique and can detect ASFV DNA from a very early stage of infection in
51. tissues, ethylene diamine tetra-acetic acid (EDTA)-blood and serum samples. The PCR is particularly useful if samples
52. submitted are unsuitable for virus isolation and antigen detection because they have undergone putrefaction. Pigs that
53. have recovered from acute, subacute or chronic infections usually exhibit a viraemia for several weeks making the PCR
54. test a very useful tool for the detection of ASFV DNA in pigs infected with low or moderately virulent strains. Virus isolation
55. by the inoculation of pig leukocyte or bone marrow cultures and identification by haemadsorption tests (HAD) are
56. recommended as a confirmatory test when ASF is positive by other methods, particularly in the event of a primary outbreak
57. or a case of ASF.
58. ~~As no vaccine is available, the presence of ASFV antibodies is indicative of previous infection and, as antibodies are~~
59. ~~produced from the first week of infection and persist for long periods, they are a good marker for the diagnosis of the~~
60. ~~disease, particularly in subacute and chronic forms.~~
61. Vaccines should be prepared in accordance with Chapter 1.1.8 *Principles of veterinary vaccine production*. ASF modified
62. live virus (MLVs) vaccines are based on the live virus that have been naturally attenuated or attenuated by targeted genetic
63. recombination through cell cultures (Gladue & Borca, 2022). MLV production is based on a seed-lot system consistent with
64. the *European Pharmacopoeia* (11th edition) and that has been validated with respect to virus identity, sterility, purity,
65. potency, safety, non-transmissibility, stability and immunogenicity. ASF MLV first generation vaccines – defined as those
66. for which peer-reviewed publications are in the public domain – should meet or exceed the minimum standards as
67. described below. Paramount demonstration of acceptable safety and efficacy against the epidemiologically relevant ASFV
68. field strain(s) where the vaccine is intended for use are required. At the present time, acceptable efficacy should be shown
69. against the B646L (p72) genotype II pandemic virus lineage currently circulating widely in domestic pigs and wild boar.
70. ASF MLV first generation vaccines allowing the differentiation of infected animals from vaccinated animals (DIVA) by
71. suitable methods (e.g. serology-based tests) are preferred. Demonstration of MLV safety and efficacy in breeding-age
72. boars, gilts and pregnant sows, and onset and duration of protective immunity, are also preferred but are not required to
73. meet the minimum standard.
74. ASF epidemiology is complex with different epidemiological patterns of infection occurring in Africa and Europe. ASF
75. occurs through transmission cycles involving domestic pigs, wild boar, wild African suids, and soft ticks (Sánchez-Vizcaíno
76. *et al.,* 2015). In regions where *Ornithodoros* soft-bodied ticks are present, the detection of ASFV in these reservoirs of
77. infection contributes to a better understanding of the epidemiology of the disease. This is of major importance in
78. establishing effective control and eradication programmes (Costard *et al*., 2013).
79. ASF is not a zoonotic disease and does not affect public health (Sánchez-Vizcaíno *et al.*, 2009).
80. ASFV should be handled with an appropriate level of bio-containment, determined by risk analysis in accordance with
81. Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal*
82. *facilities*.

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## 122 C. REQUIREMENTS FOR VACCINES

1. ~~At present there is no commercially available vaccine for ASF.~~
2. **1. Background**
3. The ASF p72 genotype II strain (ASFV Georgia 2007/1 lineage) (NCBI, 2020) is recognised to be the current highest global
4. threat for domestic pig production worldwide (Penrith *et al.,* 2022).
5. Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 *Principles of Veterinary Vaccine Production*.
6. Varying additional requirements relating to quality (including purity and potency), safety, and efficacy will apply in particular
7. countries or regions for manufacturers to comply with local regulatory requirements.
8. Wherever live, virulent ASFV or ASF MLVs are stored, handled and disposed, the appropriate biosecurity level, procedures
9. and practices should be used. The ASF MLV vaccine production facility should meet the requirements for containment
10. outlined in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and*
11. *animal facilities*.
12. An optimal ASF MLV first generation vaccine for the target host should have the following general characteristics (minimum
13. standards):
14.  Safe: demonstrate absence of fever and clinical signs of acute or chronic ASF in vaccinated and in-contact animals,
15. minimal and ideally no vaccine virus transmission, and absence of an increase in virulence (genetic and phenotypic
16. stability);
17.  Efficacious: protects against mortality, reduces acute disease (fever accompanied by the appearance of clinical signs
18. caused by ASF) and reduces vertical (boar semen and placental) and horizontal disease transmission;
19.  Quality – purity: free from wild-type ASFV and extraneous microorganisms that could adversely affect the safety,
20. potency or efficacy of the product;
21.  Quality – potent: the log10 virus titre maintained throughout the vaccine shelf life that guarantees the efficacy
22. demonstrated by the established minimum immunising (protective) dose.
23.  Identity: based on the capacity to protect against the ASFV B646L (p72) genotype II pandemic strain or other p72
24. genotypes of recognised epidemiologic importance.
25. Vaccine production should be carried out using a validated, controlled and consistent manufacturing process.
26. ASF MLV first generation vaccines must be safe (i.e. an acceptable safety profile) for non-target species and the
27. environment in general.
28. Ideally, ASF MLV first generation vaccines that meet the minimum standards should also fulfil the following additional
29. general characteristics: i) prevents acute and persistent (carrier state) disease; ii) prevents horizontal and vertical disease
30. transmission; iii) induces rapid protective immunity (e.g. < 2 weeks); and iv) confers stable, life-long immunity.
31. Furthermore, ASF MLV second and future generation vaccines should meet the minimum safety and efficacy standards
32. as ASF MLV first generation vaccines, and ideally provide additional product profile benefits, including but not limited to: i)
33. contain a negative marker allowing the differentiation of infected from vaccinated animals (DIVA) by reliable discriminatory
34. tests such as serology-based tests; and ii) confer broad range of protection against other p72 genotype field strains of
35. varying virulence (low, moderate, and high).
36. The majority of ASF global vaccine research groups and companies are currently focused on ASF MLV first generation
37. vaccine candidates that are safe and efficacious against ASF viruses belonging to the ASFV p72 genotype II pandemic
38. strain (ASFV Georgia 2007/1 lineage) (NCBI, 2020).
39. Currently, two gene deleted MLV recombinant vaccines (ASFV-G-ΔI177L and ASFV-G-ΔMGF) have been licenced for
40. field use in Vietnam following supervised field testing to evaluate the safety and effectiveness of several vaccine batches.
41. There are numerous, promising ASF MLV vaccine candidates targeting the p72 genotype II pandemic strain under
42. development, including:
43.  A naturally attenuated field strain (Lv17/WB/Rei1) (Barasona *et al.,* 2019) being developed as an oral bait vaccine for
44. wild boars;
45.  A laboratory thermo-attenuated field strain (ASFV-989) (Bourry *et al.,* 2022);
46.  Single gene-deleted, recombinant viruses (e.g. SY18ΔI226R, ASFV-G-ΔA137R) (Gladue *et al*., 2021; Zhang *et al*.,

169 2021);

1.  Double gene-deleted, recombinant viruses (e.g. ASFV-G-Δ9GL/ΔUK; ASFV-SY18-∆CD2v/UK; Arm-ΔCD2v-ΔA238L)
2. (O’Donnell *et al*., 2016; Pérez-Núñez *et al*., 2022; Teklue *et al*., 2020);
3.  Multiple gene-deleted, recombinant viruses (ASFV-G-ΔI177L/ΔLVR; ASFV-G-ΔMGF; BA71ΔCD2; HLJ/18-7GD;
4. ASFVGZΔI177LΔCD2vΔMGF) (Borca *et al*., 2021; Chen *et al*., 2020; Liu *et al*., 2023; Monteagudo *et al*., 2017;
5. O’Donnell *et al*., 2015).
6. Information regarding many of these MLV vaccine candidates can be found in a recent review publication (Brake, 2022).
7. Different DIVA strategies using serological methods (e.g. ELISA) or genome detection methods (e.g. differential real-time
8. PCR) are not widely available for these ASF MLV first generation vaccine candidates. Therefore, there is still room for
9. improvement with respect to marker vaccines and their companion diagnostic tests.
10. Inactivated (non-replicating) whole virus vaccines are not presently available and may be difficult to develop to meet
11. minimum efficacy standards. Recombinant vectored, subunit vaccine candidates that can be produced in scalable vaccine
12. platform expression systems and mRNA-based ASF vaccines are being evaluated in ongoing laboratory research, testing
13. and evaluation in experimental challenge models*.* The publicly available *Center of Excellence for African Swine Fever*
14. *Genomics* (ASFV Genomics, 2022[43](#_bookmark128)*)* that provides the structural protein predictions for all 193 ASFV proteins may help
15. accelerate ASF first and second generation vaccine research and development.
16. Fit-for-purpose vaccine use scenarios matched to the intended use in a domestic pig specific type of production system
17. may require different vaccine product profiles or may influence the focus of essential versus ideal vaccine requirements.
18. As with any MLV vaccine, all ASF MLV vaccines should be used according to the label instructions, under the strict control
19. of the country’s Regulatory Authority.
20. The minimum standards given here and in chapter 1.1.8 are intended to be general in nature and may be supplemented
21. by national, regional, and veterinary international medicinal product harmonised requirements. Minimum data requirements
22. for an authorisation in exceptional circumstances should be considered where applicable.
23. **2. Outline of production and minimum requirements for vaccines**

##### 2.1. Characteristics of the seed

1. 2.1.1. Biological characteristics of the master seed
2. MLVs are produced from ASFV field strains derived from naturally attenuated field isolates or using
3. DNA homologous (genetically targeted) recombination techniques in cell cultures to delete one or
4. more ASFV genes or gene families. These molecular techniques typically involve replacement of the
5. targeted ASFV gene(s) with one or more positive, marker fluorescent (e.g. BFP, eGFP, mCherry) or
6. enzyme-based (e.g. β-glucuronidase) ASFV promoter-reporter gene systems that allow the use of
7. imaging microscopy or flow cytometry to visualise, select, and clone gene-deleted, recombinant, ASF
8. MLVs. MLV production is carried out in cell cultures based on a seed-lot system.
9. Master seed viruses (MSVs) for MLVs should be selected and produced based on their ease of
10. growth in cell culture, virus yield (log10 infectious titre) and genetic stability over multiple cell
11. passages. Preferably, a continuous well-characterised cell line (e.g. ZMAC-4; PIPEC; IPKM) (Borca
12. *et al*., 2021; Masujin *et al*., 2021; Portugal *et al*., 2020) is used to produce a master cell bank (MCB)
13. on which the MSV and MSV-derived working seed virus (WSV) can be produced. The exact source
14. of the underlying ASFV isolate, the whole genome sequence, and the passage history must be
15. recorded.
16. 2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)
17. Only MSVs that have been established as sterile, pure (free of wild-type parental virus and free of
18. extraneous agents as described in Chapter 1.1.9 *Tests for sterility and freedom from contamination*
19. *of biological materials intended for veterinary use*, and those listed by the appropriate licensing
20. authorities) and immunogenic, should be used as the vaccine virus (WSV and vaccine batch
21. production). Live vaccines must be shown not to cause disease or other adverse effects in target
22. animals in accordance with chapter 1.1.8, Section 7.1 *Safety tests* (for live attenuated MSVs), that
23. includes target animal safety tests, increase in virulence tests, assessing the risk to the environment)
24. and if possible, no transmission to other animals.
25. Identity of the MSV must be confirmed using appropriate methods (e.g. through the use of vaccine
26. strain-specific whole genome detection methods such as next generation sequencing).
27. Demonstration of MSV stability over several cell passages is necessary, typically through at least five
28. passages (e.g. MSV+5). For those MLV vaccines for which attenuation is linked to specific
29. characteristics (gene deletion, gene mutations, etc.), genetic stability of attenuation throughout the
30. production process should be confirmed using suitable methods. Suitable techniques to demonstrate
31. genetic stability may include but are not limited to: genome sequencing, biochemical, proteomic,
32. genotypic (e.g. detection of genetic markers) and phenotypic strain characterisation. If final product
33. yields (infectious titres) are relatively low, genetic stability at a minimum of MSV+10 should be
34. demonstrated to allow more flexibility in the outline of production. For example, if MSV+8 is the
35. maximum passage for use in final product manufacturing, demonstration of genetic stability to at least
36. MSV+10 is warranted.

1. [http://asfvgenomics.com](http://asfvgenomics.com/). Accessed 4/4/2023.
2. 2.1.3. Validation as a vaccine strain
3. The vaccine derived from the MSV must be shown to be satisfactory with respect to safety and
4. efficacy.
5. Even if pigs are not known for susceptibility to transmissible spongiform encephalopathy (TSE)
6. agents, consideration should also be given to minimising the risk of TSE transmission by ensuring
7. that animal origin materials from TSE-relevant species, if no alternatives exist for vaccine virus
8. propagation, comply with the measures on minimising the risk of transmission of TSE.
9. Ideally, the vaccine virus in the final product should generally not differ by more than five passages
10. from the master seed lot.
11. ASF vaccine should be presented in a suitable pharmaceutical form (e.g. lyophilisate or liquid form).

##### 2.2. Method of manufacture

1. 2.2.1. Procedure
2. The MLV virus is used to infect swine primary cell cultures obtained from specific-pathogen free pigs,
3. the requirements for which are defined in specific monographs (Chapter 2.3.3 *Minimum requirements*
4. *for the organisation and management of a vaccine manufacturing facility*, Section 2.4.2). Compared
5. with primary cell cultures, use of a continuous cell line generally allows for more consistency, higher
6. serial volumes in manufacturing and aligns better with a seed lot system. Thus, preferably a master
7. cell bank based established, continuous cell line shown to support genetically stable ASFV replication
8. and acceptable titres over several passages should be used.
9. Cell cultures shall comply with the requirements for cell cultures for production of veterinary vaccines
10. in chapter 1.1.8. Regardless of the production method, the substrate should be harvested under
11. aseptic conditions and may be subjected to appropriate methods to release cell-associated virus (e.g.
12. freeze–thaw cycles, detergent lysis). The harvest can be further processed by filtration and other
13. purification methods. A stabiliser or other excipients may be added as appropriate. The vaccine is
14. homogenised to ensure a uniform batch/serial.
15. 2.2.2. Requirements for ingredients
16. All ingredients used for vaccine production should be in line with requirements in chapter 1.1.8.
17. 2.2.3. In-process controls
18. In-process controls will depend on the protocol of production: they include virus titration of bulk
19. antigen and sterility tests.
20. 2.2.4. Final product batch tests
21. i) Sterility
22. Tests for sterility and freedom from contamination of biological materials intended for veterinary use
23. may be found in chapter 1.1.9.
24. ii) Identity
25. Appropriate methods such as specific genome detection methods (e.g. specific differential real-time
26. 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.

**RATIONLE:** This phrase is added as explanatory to clarify the goal of the parenthetical (e.g., specific differential real-time PCR). Due to the genome size and methods of the vaccine virus generation, there is a biological (i.e., cell culture) phase present in every pathway of mutant generation in the lab. This leads to the potential for contamination with other viruses and the potential for contamination with parental (i.e., wild type, virulent) virus in the vaccine virus cultures.

1. iii) Purity
2. Appropriate methods should be used to ensure that the final product batch does not contain any
3. residual wild-type ASFV.
4. iv) Safety
5. Batch safety testing is to be carried out unless consistent safety of the product is demonstrated and
6. approved in the registration dossier and the production process is approved for consistency in
7. accordance with the standard requirements referred to in chapter 1.1.8.
8. v) Batch/serial potency
9. Virus titration is a reliable indicator of vaccine potency once a relationship has been established
10. between the vaccine minimum immunising dose (MID) (minimum protective dose) and titre of the
11. modified live vaccine *in vitro*. In the absence of a demonstrated correlation between the virus titre
12. and protection, an efficacy test will be necessary (Section C.2.3.3 *Efficacy requirements*, below).
13. vi) Residual humidity/residual moisture
14. The test should be carried out consistent with VICH [44](#_bookmark129) GL26 (*Biologicals: Testing of Residual*
15. *Moisture*, 2003[45](#_bookmark130)). Required for MLV vaccines presented as lyophilisates for suspension for injection.

##### 2.3. Requirements for authorisation/registration/licensing

1. 2.3.1. Manufacturing process
2. For regulatory approval of a vaccine, all relevant details concerning history of the pre-MSV,preparation of MSV, manufacture of the vaccine and quality control testing (Sections C.2.1
3. *Characteristics of the seed* and C.2.2 *Method of manufacture*) should be submitted to the authorities.
4. Information shall be provided from three consecutive vaccine batches originating from the same MSV
5. and representative of routine production, with a volume not less than 1/10, and more preferably with
6. a volume not less than 1/3 of the typical industrial batch volume. The in-process controls are part of
7. the manufacturing process.
8. 2.3.2. Safety requirements
9. For the purpose of gaining regulatory approval, the following safety tests should be performed
10. satisfactorily.
11. As a minimum standard, vaccines should be tested for any pathogenic effects on healthy domestic
12. pigs of the target age intended for use. Additional demonstration of MLV safety in breeding age gilts
13. and pregnant sows is preferred but not required as a minimum standard.
14. i) Safety in young animals
15. Carry out the test by each recommended route of administration using, in each case, piglets a
16. minimum of 6-weeks old and not older than 10-weeks old.
17. The test is conducted using no fewer than eight healthy piglets, and preferably no fewer than ten
18. healthy piglets.
19. Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine.
20. Administer to each piglet a quantity of the vaccine virus equivalent to not less than ten times the
21. maximum virus titre (e.g. 50% haemadsorption dose [HAD50], 50% tissue culture infective dose
22. [TCID50], quantitative PCR, etc.) (maximum release dose) likely to be contained in one dose of the
23. vaccine. To obtain individual and group mean baseline temperatures, the body temperature of each
24. vaccinated piglet is measured on at least the 3 consecutive days preceding administration of the
25. vaccine.
26. To confirm the presence or absence of fever accompanied by acute and chronic disease, observe
27. the piglets 4 hours after vaccination and then at least once daily for at least 45 days, preferably 60
28. days post-vaccination. Carry out the daily observations for signs of acute and chronic disease using
29. a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.,*
30. 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or
31. cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive
32. ﬁndings).
33. At a minimum of 45 days post-vaccination, humanely euthanise all vaccinated piglets. Conduct gross
34. pathology on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes
35. VICH: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products
36. https://[www.ema.europa.eu/en/documents/scientific-guideline/vich-gl26-biologicals-testing-residual-moisture-step-7\_en.pdf](http://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl26-biologicals-testing-residual-moisture-step-7_en.pdf)
37. (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular
38. nodes).
39. The vaccine complies with the test if:
40.  No piglet shows abnormal (local or systemic) reactions, reaches the pre-determined humane
41. endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;
42.  The average body temperature increase for all vaccinated piglets (group mean) for the
43. observation period does not exceed 1.5°C above baseline; and no individual piglet shows a
44. temperature rise above baseline greater than 2.5°C for a period exceeding 3 days.
45.  No vaccinated pigs show notable signs of disease by gross pathology
46. ii) Safety test in pregnant sows and test for transplacental transmission
47. There is currently an absence of published information on ASFV pathogenesis in breeding-age gilts
48. and in pregnant sows associated with ASFV transplacental infection and fetus abortion/stillbirth. If a
49. label claim is pursued for use in breeding age gilts and sows, then a safety study in line with VICH
50. GL44 (*Guidelines on Target Animal Safety for Veterinary Live and Inactivated Vaccines*, *Section 2.2.*
51. *Reproductive Safety Test*, 2009 [46](#_bookmark131)) should be completed.
52. iii) Horizontal transmission
53. The test is conducted using no fewer than 12 healthy piglets, a minimum of 6-weeks old and not older
54. than 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood
55. samples are negative on real-time PCR. All piglets are housed,, together from day 0 and the number
56. of vaccinated animals is the same as the number of naïve, contact animals. Co-mingle equal numbers
57. of vaccinated and naïve, contact piglets in the same pen or room.
58. Use vaccine virus at the least attenuated passage level that will be present between the master seed
59. lot and a batch of the vaccine. Administer by each recommended route of administration to no fewer
60. than six piglets a quantity of the vaccine virus equivalent to not less than the maximum virus titre
61. (maximum release dose) likely to be contained in 1 dose of the vaccine.
62. To obtain individual and group mean baseline temperatures, the body temperature of each naïve,
63. contact piglet is measured on at least the 3 consecutive days preceding co-mingling with vaccinated
64. piglets. The body temperature of each naïve, contact piglet is then measured daily for at least 45
65. days, preferably 60 days.
66. To confirm the presence or absence of fever accompanied by disease, observe the naïve, contact
67. piglets daily for at least 45 days, preferably 60 days. Carry out the daily observations for signs of
68. acute and chronic clinical disease using a quantitative clinical scoring system adding the values for
69. multiple clinical signs (e.g. Gallardo *et al.,* 2015a). These clinical signs should include fever, anorexia,
70. recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints,
71. respiratory distress and digestive ﬁndings.
72. In addition, blood should be taken from the naïve contact piglets at least twice a week for the first 21
73. days post-vaccination and then on a weekly basis. From the blood samples, determine infectious
74. virus titres by quantitative virus isolation (HAD50/ml or TCID50/ml) and using a real-time PCR test. If
75. the vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test
76. only may be used.
77. Collect blood (serum) samples from the naïve contact pigs at least at day 21 and day 28 days and
78. carry out an appropriate test to detect vaccine virus antibodies. At a minimum of 45 days, humanely
79. euthanise all naïve, contact piglets. Conduct gross pathology on spleen, lung, tonsil, and kidney
80. tissue samples and at least three different lymph nodes. Determine virus titres in all collected samples
81. by quantitative virus isolation (HAD50/mg or TCID50/mg) and real-time(RT)-PCR (see Section B.1.
82. Identification of the agent). If the vaccine virus is non-haemadsorbing or does not cause cytopathic
83. effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection)
84. may be used.

1. [https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl44-target-animal-safety-veterinary-live-inactived-vaccines-step-](https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl44-target-animal-safety-veterinary-live-inactived-vaccines-step-7_en.pdf) [7\_en.pdf.](https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl44-target-animal-safety-veterinary-live-inactived-vaccines-step-7_en.pdf)
2. The vaccine complies with the test if:
3.  No vaccinated or naïve contact piglet shows abnormal (local or systemic) reactions, reaches the
4. predetermined humane endpoint defined in the clinical scoring system or dies from causes
5. attributable to the vaccine;
6.  The average body temperature increase for all naïve, contact piglets (group mean) for the
7. observation period does not exceed 1.5°C: above baseline; and no individual piglet shows a
8. temperature rise above baseline greater than 2.5°C for a period exceeding 3 days;
9.  No naïve, contact piglet shows notable signs of disease by gross pathology and no virus is
10. detected in their blood or tissue samples by virus isolation or real-time PCR.
11. **RATIONALE:** It is important to be precise in acceptance criteria
12.  No naïve contact pigs test positive for antibodies to the vaccine virus.
13. iv) Post-vaccination kinetics of viral replication (MLV blood and tissue dissemination) study
14. Prior to the reversion to virulence study (Section C2.3.2.v. below), a minimum of one study should be
15. performed to determine the post-vaccination kinetics of virus replication in the blood (viremia), tissues
16. and viral shedding.
17. The test consists of the administration of the vaccine virus from the master seed lot to no fewer than
18. eight healthy piglets, and preferably ten healthy piglets, a minimum of 6-weeks old and not older than
19. 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood samples
20. are negative on real-time PCR.
21. Administer to each piglet, using the recommended route of administration most likely to result in
22. spread (such as the intramuscular route or intranasal route), a quantity of the master seed vaccine
23. virus equivalent to not less than the maximum virus titre (maximum release dose) likely to be
24. contained in 1 dose of the vaccine.
25. Record daily body temperatures and observe inoculated animals daily for clinical disease for at least
26. 45 days, preferably 60 days.
27. Carry out the daily observations for signs of acute and chronic clinical disease using a quantitative
28. clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo *et al.* (2015a). These
29. clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint
30. swelling and necrotic lesions around the joints, respiratory distress and digestive ﬁndings.
31. Collect blood samples from all the piglets at least two times per week from 3 days post-vaccination
32. for the first 2 weeks, then weekly for the duration of the test. Determine vaccine virus titres by
33. quantitative virus isolation (HAD50/ml or TCID50/ml) and using a real-time PCR test. If the vaccine
34. virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only may be
35. used.
36. Determine which blood timepoint(s) should be used in the design of the reversion to virulence study
37. (Section C2.3.2.v. below).Collect oral, nasal and faecal swab samples (preferably devoid of blood to
38. minimise assay interference) at least two times per week from 3-days post-vaccination for the first
39. 2 weeks, then weekly for the duration of the test. Test the swabs for the presence of vaccine virus.
40. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/ml or TCID50/ml)
41. and using a real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause
42. cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT
43. detection) may be used.
44. Euthanise at least two piglets on days 7, 14, 21, and preferably on day 28 (±2 days at each timepoint)
45. and collect spleen, lung, tonsil, kidney tissue samples and at least three different lymph nodes (which
46. should include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes).
47. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg)
48. and using real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic
49. effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT detection)
50. may be used.
51. Determine which tissue(s) and timepoint(s) should be used to aid in the design of the reversion to
52. virulence study (Section C.2.3.2.v), for example, specific tissues at specific timepoints which show
53. the highest titres should be considered for selection and use in the reversion to virulence study.
54. v) Reversion to virulence
55. The test should be carried out consistent with VICH GL41 (Examination of live veterinary vaccines in
56. target animals for absence of reversion to virulence, 2008[47](#_bookmark132)).
57. The test for increase in virulence consists of the administration of the vaccine master seed virus to
58. healthy piglets of an age (e.g. between 6-weeks and 10-weeks old) suitable for recovery of the strain
59. and of the same origin, that do not have antibodies against ASFV, and blood samples that are
60. negative on real-time PCR. This protocol is typically repeated five times.
61. *First pass* (*p1*)
62. Administer to no fewer than two piglets, and preferably no fewer than four piglets using the intended
63. route of administration for the final product, a quantity of the master seed vaccine virus equivalent to
64. not less than the maximum virus titre (maximum release dose) likely to be contained in 1 dose of the
65. vaccine. Observe inoculated animals daily for the appearance of at least two and preferably at least
66. three clinical signs and record daily body temperatures.
67. Based on results from at least one completed vaccine shed and spread (virus blood and tissue
68. dissemination study, Section C.2.3.2.iv above) collect an appropriate quantity of blood or tissue from each piglet on the predetermined single timepoint (day 5–13). Determine virus titres in individual blood samples by quantitative virus isolation (HAD50/ml or TCID50/ml) 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 sample(s) with the highest infectious titre and reserve for the subsequent *in-vivo* passage (second pass, p2).
69. Based on results from at least one completed vaccine virus blood and tissue distribution
70. dissemination study, Section C.2.3.2.iv above) euthanise piglets on the predetermined timepoint (i.e.
71. day 7, 14, 21, or 28). Determine infectious virus titres in individual blood and tissue samples by quantitative
72. virus isolation (HAD50/ml or TCID50/ml). If the vaccine virus is non-haemadsorbing or does not cause
73. cytopathic effects, a real-time PCR test or other appropriate method (e.g. titration using IPT or FAT
74. detection) may be used. Identify individual ~~tissue~~ sampletype(s) with the highest infectious titre. Pool the blood/tissues from different organs from all animals with the highest titres and prepare at least a 10% suspension in PBS, pH 7.2 kept at 4°C or at –70°C for longer storage. Test each ~~blood and tissue~~ sample pool used for inoculation by PCR to confirm the absence of potential viral agent contaminants (i.e.,FMDV, PRRS, PCV2). Blood and pooled tissue (p1) are used to inoculate 2 ml of positive material using the intended route of administration for the final product to each of least two and ideally at least four further pigs of the same age and origin

**RATIONALE:** ASFV is recognized in several tissues in swine and is noted in this section above. Several edits provided as consistent statements that both blood and tissue are needed for studies. In fact, epithelial tissues, not blood, is not likely to cause disease transmission to other pigs. <https://www.cfsph.iastate.edu/Factsheets/pdfs/african_swine_fever.pdf>

1. *Second pass* (*p2*)
2. If no virus is found (p1), repeat the administration by the intended route once again with the same
3. pooled material (blood and pooled tissue, p1) in another ten healthy piglets of the same age and
4. origin.
5. If no virus is found at this point, end the process here. If, however, virus is found, carry out a second
6. series of passages by administering 2 ml of positive material using the intended route of
7. administration for the final product to each of no fewer than two piglets, and preferably no fewer than
8. four piglets of the same age and origin. Observe inoculated animals daily for the appearance of at
9. least two and preferably at least three clinical signs and record daily body temperatures.
10. *Third and fourth pass* (*p3 and p4*)
11. If no virus (p2), repeat the intramuscular administration once again with the same pooled material
12. (blood and pooled tissue, p2) in another eight healthy piglets of the same age and origin.
13. If no virus is found at this point, end the process here. If, however, virus is found, carry out this
14. passage operation no fewer than two additional times (p3 and p4) (to each of no fewer than two
15. piglets, and preferably no fewer than four piglets of the same age and origin) and verifying the
16. presence of the virus at each passage in blood and tissues. Observe inoculated animals daily for the

1. [https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl41-target-animal-safety-examination-live-veterinary-vaccines-](https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl41-target-animal-safety-examination-live-veterinary-vaccines-target-animals-absence-reversion_en.pdf) [target-animals-absence-reversion\_en.pdf.](https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl41-target-animal-safety-examination-live-veterinary-vaccines-target-animals-absence-reversion_en.pdf)
2. appearance of at least two and preferably at least three clinical signs and record daily body
3. temperatures.
4. *Fifth pass* (*p5*)
5. Administer 2 ml of the blood and pooled tissue (4) to each of at least eight healthy piglets of the same
6. age and origin. Observe inoculated animals daily for at least 28 days post-inoculation for the
7. appearance of at least two and preferably at least three clinical signs, and daily body temperature.
8. The vaccine virus complies with the test if:
9.  No piglet shows abnormal local or systemic reaction, reaches the pre-determined humane end
10. point defined in the clinical scoring system or dies from causes attributable to the vaccine; and
11.  There is no indication of increasing virulence (as monitored by daily body temperature
12. accompanied by clinical sign observations) of the maximally passaged virus compared with the
13. master seed virus.
14. At a minimum, a safe MLV vaccine shall demonstrate ALL the following features (minimal standards):
15. • Absence of fever (defined as average body temperature increase for all vaccinated piglets (group
16. mean) for the observation period does not exceed 1.5°C above baseline; and no individual piglet
17. shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days);
18.  Absence of chronic and acute clinical signs and gross pathology over the entire test period or
19. minimal chronic clinical signs (defined as mild swollen joints with a low clinical score that resolve
20. within 1 week).
21. • Minimal (defined as no naïve, contact piglet shows notable signs of disease by clinical signs and
22. gross pathology and no or a low percentage of contact piglets test both real-time PCR positive
23. and seropositive) or no vaccine virus transmission (defined as no naïve, contact piglet shows
24. notable signs of disease by clinical signs and gross pathology and no contact piglets test both
25. real-time PCR positive and seropositive) over the entire test period;
26. • Absence of an increase in virulence (genetic and phenotypic stability) (complies with the reversion
27. to virulence test).
28. In addition, the vaccines in their commercial presentation before being authorised for general use
29. should be tested for safety in the field (see chapter 1.1.8 Section 7.2.3). Additional field safety
30. evaluation studies may include but are not limited to: environmental persistence (e.g. determination
31. of virus recovery from bedding or other surfaces), assessment of immunosuppression, and negative
32. impacts on performance.
33. 2.3.3. Efficacy requirements
34. i) Protective dose
35. Vaccine efficacy is estimated in immunised animals directly, by evaluating their resistance to live
36. virus challenge. The test consists of a vaccination/challenge trial in piglets a minimum of 6-weeks old
37. and not more than 10-weeks old, free of antibodies to ASFV, and negative blood samples by real-
38. time PCR. The test is conducted using no fewer than 15 and preferably no fewer than 24 vaccinated
39. pigs, and no fewer than five non-vaccinated control piglets.
40. The test is conducted to determine the minimal immunising dose (MID) (also referred to as the
41. minimal protective dose [MPD] or protective fraction); using at least three groups of no fewer than
42. five and preferably not fewer than eight vaccinated piglets per group, and one additional group of no
43. fewer than five non-vaccinated piglets of the same age and origin as controls. Use vaccine containing
44. virus at the highest passage level that will be present in a batch of vaccine.
45. Each group of piglets, except the control group, is immunised with a different vaccine virus content
46. in the same vaccine volume. In at least one vaccinated group, piglets are immunised with a vaccine
47. dose containing not more than the minimum virus titre (minimum release dose) likely to be contained
48. in one dose of the vaccine as stated on the label.
49. Twenty-eight days (±2 days) after the single injection of vaccine (or if using two injections of the
50. vaccine then 28 days [±2 days] following the second injection), challenge all the piglets by the
51. intramuscular route. If previous studies have demonstrated acceptable efficacy using IM challenge,
52. then a different challenge route (e.g. direct contact, oral or oronasal) may be used. Challenged,
53. vaccinated piglets may be housed in one or more separate pens in the same room or in different
54. rooms. Challenged, naïve controls can be housed in one or more rooms that are separate from
55. challenged, vaccinated piglets.
56. Carry out the test using an ASFV representative strain of the epidemiologically relevant field strain(s)
57. where the vaccine is intended for use (e.g. ASFV B646L [p72] genotype II pandemic strain and other
58. p72 virulent genotype of recognised epidemiologic importance). For gene deleted, recombinant MLV
59. viruses, if neither challenge virus type is available, then carry out the test with the parental, virulent
60. virus used to generate the MLV recombinant virus. Use a 10e3–10e4 HAD50 (or TCID50 for non-HAD
61. viruses) challenge dose sufficient to cause death or meet the humane endpoint in 100% of the
62. nonvaccinated piglets in less than 21 days. Higher or lower challenge doses can be considered if
63. appropriately justified.
64. The rectal temperature of each vaccinated piglet is measured on at least the 3 days preceding
65. administration of the challenge virus, at the time of challenge, 4 hours after challenge, and then daily
66. for at least 28 days, preferably 35 days. Observe the piglets at least daily for at least 28 days,
67. preferably 35 days. Carry out the daily observations for signs of acute and chronic clinical disease
68. using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo
69. *et al.*, 2015). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or
70. cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive
71. ﬁndings.
72. Collect blood samples from the vaccinated challenged piglets at least two times per week from 3 days
73. post-challenge for at least 28, preferably 35 days. From the blood samples, determine infectious virus
74. titres by quantitative virus isolation (HAD50/ml or TCID50/ml) and using a real-time PCR test. If the
75. vaccine virus is non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test only
76. may be used.
77. At the end of the test period, humanely euthanise all vaccinated challenged piglets. Conduct gross
78. pathology on spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes.
79. (which should include lymph node closest to site of inoculation, gastrohepatic and submandibular
80. nodes). Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or
81. TCID50/mg) and real-time PCR (see Section B.1. Identification of the agent). If the vaccine virus is
82. non-haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate
83. method (e.g. titration using IPT or FAT detection) may be used.
84. The test is invalid if fewer than 100% of control piglets die or reach a humane endpoint.
85. The vaccine (or a specific vaccine virus dose if conducting a vaccine dose titration study) complies
86. with the test if:
87.  No vaccinated challenged piglet shows abnormal (local or systemic) reactions, reaches the
88. humane endpoint or dies from causes attributable to ASF;
89.  The average body temperature increase for all vaccinated challenged piglets (group mean) for
90. the observation period does not exceed 2.0°C above baseline; and no individual piglet shows a
91. temperature rise above baseline greater than 2.0°C;
92.  The vaccinated challenged piglets display a reduction or absence of typical acute clinical signs of
93. disease and gross pathology and a reduction or absence of challenge virus levels in blood and
94. tissues.
95. ii) Assessment for horizontal transmission (challenge virus shed and spread study)
96. The ASF basic reproduction number, R0, can be defined as the average number of secondary ASF
97. disease cases caused by a single ASFV infectious pig during its entire infectious period in a fully
98. susceptible population (Hayes *et al*., 2021). In general, if the ASFV effective reproduction number
99. Re=R0 × (S/N) (S= susceptible pigs; N= total number of pigs in a given population) is greater than
100. 1.0, disease is predicted to spread. Ideally, ASF vaccination should reduce Re to less than 1.0 by
101. reducing the number of susceptible, naïve, contact pigs exposed to vaccinated, infected pigs.
102. To evaluate ASF vaccine impact on ASF disease transmission, the test consists of a
103. vaccination/challenge trial in piglets a minimum of 6-weeks old and not older than 10-weeks old, free
104. of antibodies to ASFV, and negative blood samples by real-time PCR.
105. The test is conducted using no fewer than 15 healthy piglets at a ratio comprising twice the number
106. of vaccinated piglets to naïve piglets (e.g. ten vaccinated and five naïve). Use vaccine containing
107. virus at the highest passage level that will be present in a batch of the vaccine.
108. The quantity of vaccine virus administered to each pig is equivalent to be not more than the minimum
109. virus titre (minimum dose) likely to be contained in one dose of the vaccine as stated on the label.
110. Following immunisation, vaccinated and naïve piglets should continue to be co-mingled.
111. Twenty-eight days [±2 days] after the single injection of vaccine (or if using two injections of the
112. vaccine then 28 days [± 2 days] following the second injection), temporarily separate [into different
113. pen(s) or room(s)] all vaccinated piglets from naïve piglets. Challenge all vaccinated piglets by the
114. intramuscular or other previously verified route. Carry out the challenge using an ASFV
115. representative strain of the epidemiologically relevant field strain(s) where the vaccine is intended for
116. use (e.g. ASFV B646L [p72] genotype II pandemic strain and other p72 virulent genotype of
117. recognised epidemiological importance). For gene deleted, recombinant MLV viruses, if neither
118. challenge virus type is available, then carry out the test with the parental, virulent virus used to
119. generate the MLV recombinant virus. Use a 10e3–10e4 HAD50 (or TCID50 for non-HAD viruses
120. challenge dose sufficient to cause death or met the humane endpoint in 100% of the nonvaccinated
121. piglets in less than 21 days. Higher or lower challenge doses can be considered if appropriately
122. justified.
123. Approximately 18-24 hours later, re-introduce naïve piglets to vaccinated, challenged piglets and
124. allow for direct nose to nose contact exposure with vaccinated, challenged piglets. Allow for
125. continuous contact exposure by co-mingling both groups through the end of the study. If more than
126. one pen or room is used for co-housing, following reintroduction initially maintain a ratio of 2:1 of
127. challenged, vaccinated piglets to contact exposed, naïve piglets.
128. The rectal temperature of each contact piglet is measured on at least the 3 days preceding
129. administration of the challenge virus to vaccinated pigs, immediately prior to direct contact exposure,
130. 4 hours post-contact exposure, and then daily for at least 28, preferably 35 days. Observe all contact
131. exposed piglets at least daily for at least 28 days, and preferably for at least 35 days.
132. Carry out the daily observations in each contact piglet for signs of acute and chronic clinical disease
133. using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo
134. *et al.,* 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or
135. cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive
136. ﬁndings.
137. In addition, blood should be taken from the naïve contact piglets at least twice a week from 3 days
138. post-contact exposure for the duration of the test period. From the blood samples, determine
139. infectious challenge virus titres by quantitative virus isolation (HAD50/ml or TCID50/ml) and using a
140. real-time PCR test. If the vaccine virus is non-haemadsorbing or does not cause cytopathic effects,
141. a real-time PCR test only may be used.
142. Collect blood (serum) samples from the naïve contact pigs at least at day 21 and day 28 (±2 days),
143. and at the end of the test period, and carry out an appropriate test to detect vaccine virus antibodies.
144. Collect oral, nasal and faecal swab samples (preferably devoid of blood to minimise assay
145. interference) from all contact-exposed naïve piglets at least two times per week from 3-days post-
146. contact exposure for the first 2 weeks, then weekly for the duration of the test and test swabs for the
147. presence of challenge virus. Determine virus titres in all collected samples by quantitative virus
148. isolation (HAD50/ml or TCID50/ml) and using a real-time PCR test. If the vaccine virus is non-
149. haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate
150. method (e.g. titration using IPT or FAT detection) may be used.
151. At the end of the test period, humanely euthanise all contact piglets. Conduct gross pathology on
152. spleen, lung, tonsil, and kidney tissue samples and at least three different lymph nodes. (which should
153. include lymph node closest to site of inoculation, gastrohepatic and submandibular nodes).
154. Determine virus titres in all collected samples by quantitative virus isolation (HAD50/mg or TCID50/mg)
155. and real-time PCR (see Section B.1. Identification of the agent). If the vaccine virus is non-
156. haemadsorbing or does not cause cytopathic effects, a real-time PCR test or other appropriate
157. method (e.g. titration using IPT or FAT detection) may be used.

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| 622 | The test is invalid if the vaccine fails to comply with the compliance criteria described for the protected |
| 623 | dose test in vaccinated pigs (Section C.2.3.3.i above). |
| 624 | The vaccine complies with the test for a reduction in horizontal disease transmission if: |
| 625 | * No naïve, contact exposed piglet shows abnormal (local or systemic) reactions, reaches the |
| 626 | defined humane endpoint or dies from causes attributable to ASF; |
| 627 | * No naïve, contact exposed piglet displays fever accompanied by typical signs of disease, |
| 628 | including gross pathology. |
| 629 | * Naïve contact pigs show a reduction or absence of challenge virus levels in blood and tissues. |
| 630 | * None of or a reduced number of naïve contact exposed pigs test positive for antibodies to the |
| 631 | challenge virus. |
| 632 | At a minimum, an efficacious MLV vaccine shall demonstrate ALL the following features (minimal |
| 633 | standards): |
| 634 | * Protects against mortality; |
| 635 | * Reduces acute disease (fever accompanied by a reduction of typical clinical and pathological |
| 636 | signs of acute disease) |
| 637 | * Reduces horizontal disease transmission (no naïve, contact exposed piglet shows abnormal |
| 638 | [local or systemic] reactions, reaches the humane endpoint or dies from causes attributable to |
| 639 | ASF, and displays fever accompanied by typical acute disease signs caused by ASF) |
| 640 | * Reduces levels of viral shedding and viraemia. |
| 641 | In addition, the vaccines in their commercial presentation before being authorised for general use |
| 642 | should be tested for efficacy in the field (see chapter 1.1.8 Section 7.2.3). Additional field efficacy |
| 643 | evaluation studies may include but are not limited to: onset of immunity, duration of immunity, and |
| 644 | impact on disease transmission. |
| 645 **2.3.4.** | **Duration of immunity** |
| 646 | Although not included in the guidance for ASF MLV first generation vaccines, manufacturers are |
| 647 | encouraged as part of the authorisation procedure, to demonstrate the duration of immunity of a given |
| 648 | vaccine by evaluation of ~~potency~~ protection from disease at the end of the claimed period of protection.  **RATIONALE:** Potency is the characteristic of the vaccine, not the immune response. |
| 649 **2.3.5.** | **Stability** |
| 650 | Stability of the vaccine should be demonstrated over the shelf life recommended for the product. |
| 651 | Although not included in the standards for first generation MLV ASF vaccines, manufacturers are |
| 652 | encouraged, as part of the authorisation procedure, to generate data supporting the retention of immunogenicity over a defined period of ~~validity~~ time |
| 653 | of a lyophilised or other pharmaceutical form of the ASF vaccine as part of the authorisation |
| 654 | procedure. |

**RATIONALE:** Period of validity not defined anywhere in this document.

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816 **NB:** There are WOAH Reference Laboratories for African swine fever

817 (please consult the WOAH Web site:

818 <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

819 Please contact the WOAH Reference Laboratories for any further information on

820 diagnostic tests and reagents for African swine fever

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

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**Appendix 1 to Annex 16 of the Report of the Biological Standards Commission meeting 4–8 September 2023**

**Draft Standards for African Swine Fever (ASF) Modified Live Virus (MLV) Vaccines for Domestic and Wild Pigs**



Annex16\_3.09.01\_ap pendix1\_GL\_ASF\_MLV