VetMAX™ African Swine Fever Virus Detection Kit

TaqMan™ real-time PCR detection of African swine fever virus

Catalog Number A28809

Doc. Part No. 100027918 Pub. No. MAN0010783 Rev. D

Technology	Species	Samples	Test type
Real-time PCR (DNA) • Duplex assay • Exogenous IPC	Swine	Blood Serum	Individual Pooled samples (5 or 10 samples)
		Tissues	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Applied Biosystems™ VetMAX™ African Swine Fever Virus Detection Kit (Cat. No. A28809) enables detection of the African swine fever virus (ASFV) in swine blood, serum, or tissues by real-time PCR amplification of the ASFV P72 gene.

The assay is a single-well real-time PCR in which ASFV and exogenous Internal Positive Control (IPC) targets are amplified and detected using fluorescent TaqMan™ probes.

The kit includes:

- 3 - Mix ASFV: Contains primers, TaqMan™ probes, buffer, and enzyme for optimized duplex real-time PCR amplification of ASFV and IPC targets.
- 4a - EPC ASFV: Nucleic acid template for P72 target amplification. It serves as an external positive control for the real-time PCR reaction, and it is used to set the cycle threshold (C_t) for evaluating test results.
- 5 - IPC ASFV: Internal positive control added to each sample and control at the lysis step of the DNA extraction procedure. It serves as a control for the DNA purification process, and it is used to monitor for the presence of PCR inhibitors.

Contents and storage

Component	Amount[1]	Storage[2]
3 – Mix ASFV	2 × 1000 µL	–30°C to –10°C
4a – EPC ASFV	2 × 90 µL	
5 – IPC ASFV	1 × 500 µL	

[1] Sufficient for 100 25-µL real-time PCR reactions.
[2] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Item	Source
Applied Biosystems™ 7500 Real-Time PCR System	Contact your local sales office.
96-well plate, strip tubes (8- or 12-wells), microtubes or capillaries compatible with thermal cycler used	MLS
Nuclease-free pipettes and filtered pipette tips	MLS
Two ice buckets or refrigerated racks: • One for the PCR setup area where the PCR master mix is prepared • One for the area where DNA samples and controls are prepared	MLS
Plate covers or caps compatible with the plates, strip tubes, microtubes, or capillaries	MLS
Nuclease-free reagent tubes for preparing master mix	MLS
Nuclease-Free Water (not DEPC-Treated)	AM9939
1X TE Buffer	MLS

Procedural guidelines

- For each real-time PCR run, include the controls indicated in "Set up the PCR reactions" on page 2.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 4 to prevent false positives and contamination of test samples with PCR products.

Requirements for input DNA

We recommend using the MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359) for DNA purification from biological samples, but you can also use other high quality DNA purification methods after proper validation in your laboratory. In addition, prepare mock samples using nuclease-free water as the starting material and the same DNA purification method used for test samples.

IMPORTANT! Add 5 µL of the 5 - IPC ASFV to the lysis solution used for DNA purification for each sample and extraction control.

Before you begin

1. Thaw reagents and samples:
   a. Thaw 3 – Mix ASFV in an ice bucket or refrigerated rack.
   b. Thaw 4a – EPC ASFV, 5 – IPC ASFV, and DNA samples in a separate ice bucket or refrigerated rack.
2. Thoroughly mix the contents of each tube by vortexing, then briefly centrifuge.

Store thawed reagents, controls, and samples at 2–8°C until use.

Set up the PCR reactions

1. Dispense 20 µL of 3 – Mix ASFV to the appropriate number of PCR plate wells, strip tubes, or capillaries.
2. Add sample or control according to the following table:

Sample type	Component	Volume per reaction
Test sample	Sample DNA	5.0 µL
Positive control	4a – EPC ASFV	5.0 µL
Extraction control	Mock sample	5.0 µL
No-template control (NTC)	Nuclease-free water	5.0 µL

3. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
   - Reaction volume: 25 µL
   - Passive reference: ROX™ dye (included in 3 – Mix ASFV)

     Note: ROX™ dye must be set up if the instrument is capable of detecting it. Real-time PCR instruments that do not detect ROX™ dye can be used without affecting the accuracy of the reading.
   - Select detectors and assign TaqMan™ probe reporter dyes and quenchers for each well, tube, or capillary used in the analysis.

Target	Reporter	Quencher
ASFV	FAM™ dye	Non-fluorescent quencher (NFQ)
IPC	VIC™ dye	TAMRA™ dye[1]

[1] TAMRA™ dye must be set up for real-time PCR analysis if the instrument is capable of detecting it. Real-time PCR instruments that do not detect TAMRA™ dye can be used without affecting the accuracy of the reading.

   - Thermal cycling program:

Stage	Repetitions	Temperature	Time
1	1	50°C	2 minutes
2	1	95°C	10 minutes
3	45	95°C	15 seconds
		60°C	1 minute

2. Run the thermal cycler program, collecting real-time amplification data during stage 3.

Guidelines for data analysis

- Follow the instrument user guide for raw data analysis.
- Set the thresholds for each target separately.
- Interpret the results for each control and sample according to the obtained C_t values as indicated in the following sections.

Validation criteria

Refer to the C_{tQC} values in the Certificate of Analysis for the manufacturing lot of the kit. The test is validated if the following criteria are met:

Reaction type	ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC}$ ASFV ± 3 C_t[1]	$C_t < 45$ or $C_t > 45$[2]	PCR is validated.
Extraction control[3]	$C_t > 45$	$C_t = C_{tQC}$ IPC ± 3 C_t[4]	DNA extraction is validated.
No-template control	$C_t > 45$	$C_t > 45$	PCR reagents are validated.

[1] See the EPC table in the Certificate of Analysis.
[2] The IPC value of the positive control is not used for test validation.
[3] Use the extraction control prepared using the same extraction procedure as the samples.
[4] See the IPC table in the Certificate of Analysis.

Interpretation of results

ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	ASFV is detected.
$C_t > 45$	$C_t = C_t$ of extraction control ± 3 C_t [1]	ASFV is not detected.
$C_t > 45$	C_t is outside this range: C_t of extraction control ± 3 C_t[1]	Invalid result.[2]

[1] The C_t value of the extraction control must first be validated as described in “Validation criteria” on page 3.
[2] The result is invalid due to a non-compliant IPC result.

Retest samples with invalid results

1. Dilute the DNA samples 1:10 in 1X TE buffer.
2. Repeat the real-time PCR procedure with 5 µL of the diluted DNA, then interpret the results as follows.

Result	Interpretation
The diluted DNA is positive for ASFV.	The result is validated.
The diluted DNA is negative for ASFV, and the IPC result is compliant.	
The diluted DNA is negative for ASFV, but the IPC result is non-compliant.	The result is invalid.

3. For diluted samples with invalid results, repeat the DNA extraction procedure on a new aliquot of the original sample lysate, then repeat the test.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

World Organisation for Animal Health (WOAH) Certification

Validated and certified by the WOAH as fit for the purposes defined in the kit insert. Registration number: 20200114.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0010783 D

Revision	Date	Description
D	10 February 2025	The World Organisation for Animal Health (WOAH) logo was updated.
C.0	21 July 2020	Added the World Organisation for Animal Health (OIE) logo and registration number.
B.0	8 February 2018	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Minor edits to align with current style.
A.0	2 April 2015	Baseline for revision history.

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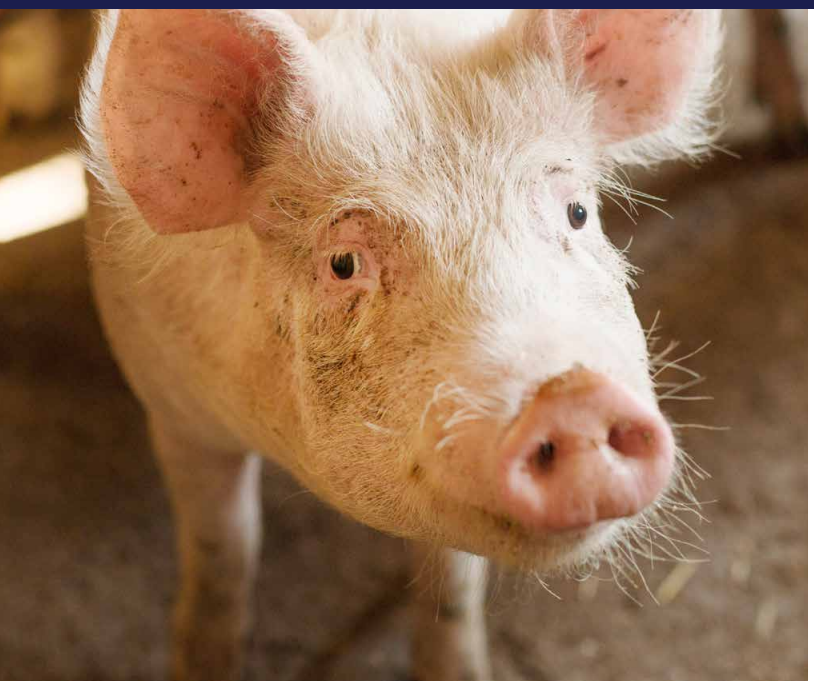

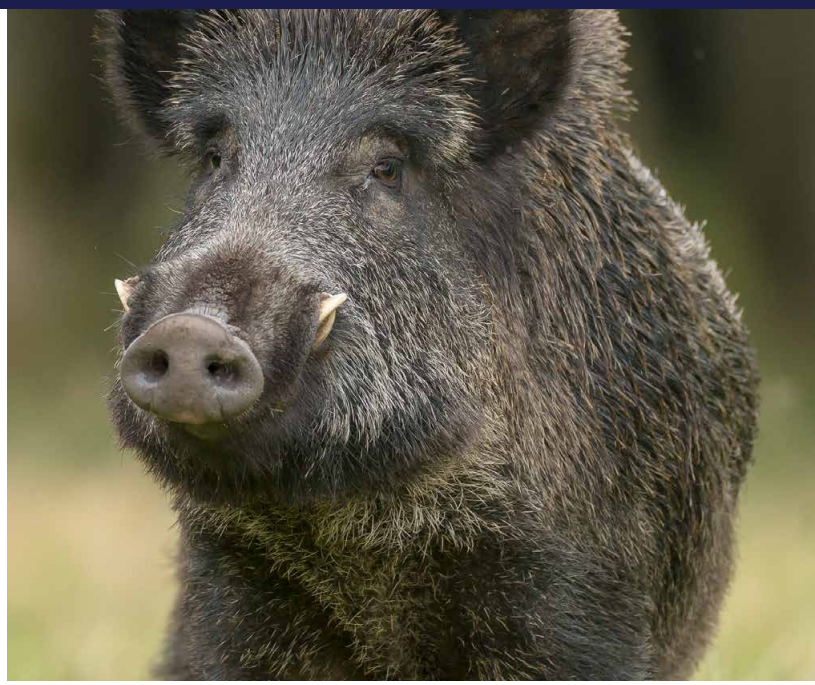

VetMAX African Swine Fever Virus Detection Kit

Real-time PCR kit—validated by the OIE* and used for virus circulation monitoring in outbreak situations

The Applied Biosystems™ VetMAX™ African Swine Fever Virus (ASFV) Detection Kit has been used since 2015 during outbreaks in Europe, Asia, and Africa for clinical confirmation and detection of ASFV in domestic and wild pigs.

Benefits

- Approved by the OIE for the detection of ASFV
- Validated by the European Union Reference Laboratory for ASFV (EURL, CISA-INIA, Spain)
- Detects all ASFV genotypes
- Allows users to test pools of up to 10 samples
- Contains a ready-to-use master mix for the detection of the ASFV target and the internal positive control (IPC)
- Delivers results in less than three hours

The VetMAX African Swine Fever Virus Detection Kit has successfully passed every step of the OIE procedure for the registration of diagnostic kits and has evidenced that it is fit-for-purpose for the detection of the African swine fever virus in blood, serum, and tissues of domestic and wild pigs (including wild boars).

Technology	Species	Samples	Test type
Real-time PCR of DNA • Duplex assay • Exogenous IPC	Domestic pig Wild boar	Blood Serum	Individual or pooled samples, up to 10
		Tissues	Individual

* World Organisation for Animal Health (OIE).

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Diagnostic sensitivity

The VetMAX African Swine Fever Virus Detection Kit has been evaluated by EURL on 424 positive samples coming from field genotype II ASFV-infected areas within Asian and European countries.

424 positive samples tested	400 positives using UPL-PCR* 94%	384 positives using the VetMAX African Swine Fever Virus Detection Kit 91%

Although the UPL-PCR test was able to detect the highest percentage of the infected animals, the kappa coefficient (κ) statistics were used to evaluate the concordance between each test, and the result of 0.87%$_{(95\%\ CI)}$ indicates **perfect agreement between the UPL reference method and the VetMAX African Swine Fever Virus Detection Kit.**

* Universal probe library (UPL) real-time PCR (Fernandez et al, 2013).

Inclusivity and exclusivity

PCR inclusivity has been evaluated on a panel of reference samples from CISA-INIA and CVI (Central Veterinary Institute, the Netherlands).

PCR exclusivity has been evaluated on various viruses, bacteria, and parasites.

	Strain	VetMAX African Swine Fever Virus Detection Kit
Inclusivity	38 reference samples from CISA-INIA	Detected
	20 reference samples from CVI	Detected
Exclusivity	Viruses (PCV1, PCV2, PPV, influenza, PRRSV, CSFV, BVDV, BHV1, porcine coronavirus, herpes virus type 1)	Not detected
	Bacteria (*Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae*, *Lawsonia* spp., *Brachyspira hyodysenteriae*, and 11 other species)	Not detected
	Parasites (*Toxoplasma gondii*, *Neospora caninum*)	Not detected

The VetMAX African Swine Fever Virus Detection Kit **correctly detected all ASF strains from CISA-INIA and CVI panels** and didn't show cross-reaction with other pathogens.

Real-time PCR workflow from sampling to result

Sample collection → **Nucleic acid extraction** → **Amplification**

Direct samples or those collected using Applied Biosystems™ GenoTube Livestock Swabs

36 min using the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit with a Thermo Scientific™ KingFisher™ instrument

90 min using the VetMAX ASFV Detection Kit with a PCR instrument such as the Applied Biosystems™ QuantStudio™ 5 system

Ordering information

Product	Quantity	Cat. No.
VetMAX African Swine Fever Virus Detection Kit	100 tests	A28809
Sample collection and sample preparation		
GenoTube Livestock Swab	1 tube	9062010
MagMAX CORE Nucleic Acid Purification Kit	500 tests	A32700

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Development and validation of African Swine Fever Real-time PCR kit

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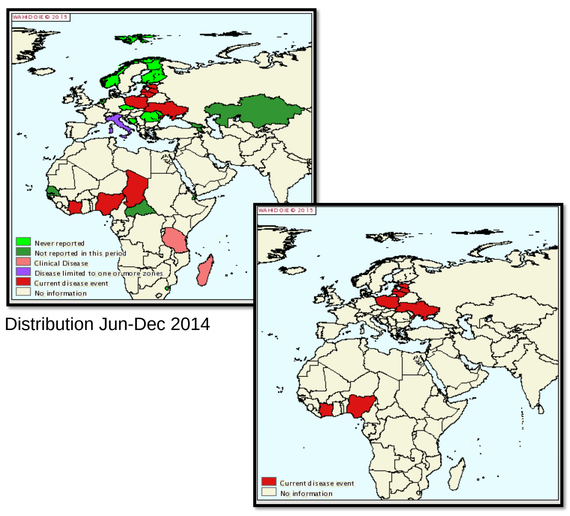
ABSTRACT

African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease that can cause significant economic losses. The disease is widely endemic in many parts of Africa, of Southern Europe and increasingly becoming a threat in Eastern Europe. As there is still no vaccine or treatment available, monitoring and controlling of the disease by means of diagnosis is the only way to control the disease and is of utmost importance. A new duplex real-time PCR kit that targets the p72 gene and an internal control has been developed and its performance for diagnosis of ASFV has been assessed. In order to demonstrate the sensitivity and specificity of the new LSI VetMAX™ African Swine Fever Virus detection kit, different internal and field studies including animal infection experiments were carried out (INIA, Spain; CVI, Netherlands; Germany). 1600 negative samples from ASFV free regions (Germany & Spain) and 33 different pathogens were tested to demonstrate specificity of the assay. About 100 ASFV positive samples from Africa and Europe were also tested. Results of the ASFV kit showed 100% sensitivity in all tested sample materials (blood, serum and tissues) and 100% specificity. No cross reaction was found with other pathogens and a serial dilution of the ASFV target sequence led to a limit of detection (LOD) of 16 genome copies per PCR reaction. The experimental LOD was 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood.
The LSI VetMAX™ African Swine Fever Virus detection kit fulfills all the validation criteria of PCR characteristics and complete method, as required by the NF U47-600-2 standard.

INTRODUCTION

African Swine Fever (ASF) is a DNA virus from the *Asfaviridae* Family. ASFV infects all *Suidae* (domestic and wild animals) but is not a human health threat. The virus is found in all body fluids and tissues of infected pigs. They usually become infected by direct contact with sick animals or by ingestion of infected products. ASFV is highly resistant in the environment. ASF disease is characterized by high fever, loss of appetite, haemorrhages in the skin and internal organs and death can occur within 2 to 10 days on average. ASF cannot be differentiated from classical swine fever by either clinical or post-mortem examination. It is an economically important disease that is widely endemic in many parts of Africa and that has become a real threat in Eastern Europe (Figure 1). In order to improve ASF diagnosis, a new duplex real time PCR kit was developed.

Figure 1. Disease Distribution maps - 2014 and 2015 (OIE)



Distribution Jun-Dec 2014

Distribution Jan-Mar 2015

MATERIALS AND METHODS

LSI VetMAX™ African Swine Fever Virus detection kit is a TaqMan™ ready-to-use real-time PCR assay based on the simultaneous detection of ASFV and an exogenous Internal Positive Control (IPC). For the development of a reliable, sensitive and specific rtPCR system, more than 450 different ASFV sequences representing the p72 protein encoding region were aligned. The isolation of viral DNA from field samples was performed with MagMax™ Pathogen RNA/DNA kit and MagVet™ Universal Isolation kit. About 1600 negative samples (blood and serum) were collected from ASFV free regions (Germany and Spain) and additionally 33 different pathogens close to ASFV or found in the same ecological niches were tested to demonstrate specificity of the assay. For validation of the sensitivity about 100 ASFV positive samples from Africa and Europe were tested. The limit of detection (LOD) was determined by serial dilution of a plasmid carrying a specific ASF sequence (pASF).

RESULTS

Table 1. Specificity of LSI VetMAX™ African Swine Fever Virus detection kit (partial data)

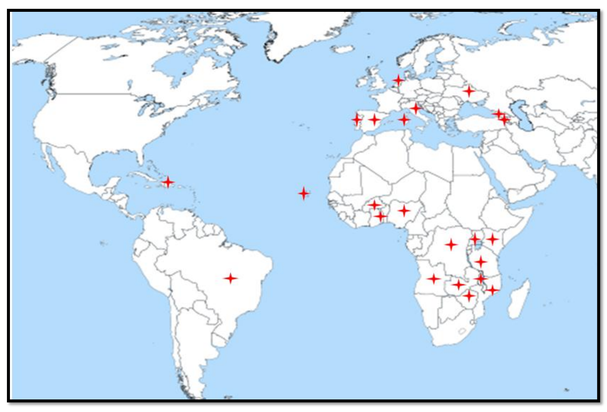
	Strain	ASFV Detection
Inclusivity	**ASFV (38 reference samples from CISA-INIA)**	Detected
	ASFV (20 strains from CVI)	Detected
Exclusivity	Classical Swine Fever Virus	Not detected
	Porcine circovirus 1	Not detected
	Porcine circovirus 2	Not detected
	Porcine Parvovirus	Not detected
	Herpes virus	Not detected
	PRRSV	Not detected
	Influenza H1N1	Not detected
	Mycoplasma hyopneumoniae	Not detected

The inclusivity of LSI VetMAX™ African Swine Fever Virus detection kit is evaluated on a panel of DNA isolated from 58 ASFV positive samples (organs and sera) coming from CISA-INIA, Spain and Central Veterinary Institute (CVI), Netherlands. As indicated in the table above, the kit show 100% inclusivity for the strains tested.

The exclusivity is assessed on a panel of 33 pathogens close to ASFV (data partially shown), either because they are preferentially found in the same ecological niches, phylogenetically close, or because they have the same clinical symptoms in target species. None of the strains tested is detected.

LSI VetMAX™ African Swine Fever Virus detection kit is specific for African Swine Fever Virus and does not detect other tested pathogens.

Figure 2. Distribution of strains tested for inclusivity



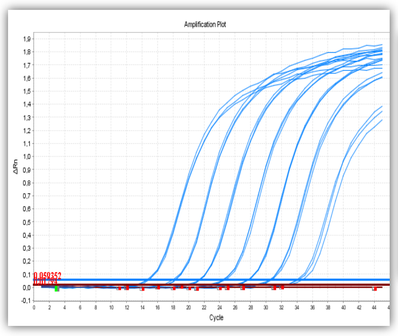
Strains and field samples tested (CISA-INIA, Spain and CVI, Netherlands) allow to recover a large distribution of the virus. All of them are detected by our PCR.

Table 2. Results obtained in ASFV positive strains (partial data)

Strain	ASFV Detection
Kat 67 - DRCongo	Detected
Malawi 82	Detected
Mozambique 64	Detected
Angola 72	Detected
Dominican Republic 80	Detected
Uganda 64	Detected
608 VR13	Detected
Lerida 1975 E75	Detected
Pontevedra 1970 E70	Detected
1207	Detected
BA71-V	Detected
L60 - Portugal	Detected
Haiti 78	Detected
Sassari 88	Detected
Dominican Republic 78	Detected
Lisbon 60	Detected
Georgia 2007	Detected
Spain - OURT 88/3	Detected
Tanzania KIRT 89/1	Detected
Zimbabwe VICT 90/1	Detected

A random set of 58 ASFV strains of different origins, including also the Georgia 2007 strain, which is representative for the ongoing outbreak in the Kaukasus, Russia, and neighbouring countries from 2007 to 2014 were tested.
All strains are detected and the results show a very high correlation between the Ct of LSI VetMAX™ African Swine Fever Virus detection kit and the in-house developed PCR in CVI or INIA (data not shown).

Figure 3. PCR efficiency of LSI VetMAX™ African Swine Fever Virus detection kit



R^2=0.998; Eff=101.5%

Table 3. Characteristics of LSI VetMAX™ African Swine Fever Virus detection kit according to AFNOR Standard for veterinary PCR (NF U47-600-2)

Characteristics	ASFV validation
Analytical specificity (Table 1)	100%
Efficiency (Figure 3)	Close to 100%
Limit of detection	16 copies per PCR
Repeatability	CV<3.01 %
Intermediate precision	CV<4.09 %
Robustness	Unaffected by all parameters tested
Experimental LOD - Serum	5x10^3 cp per mL
Experimental LOD - Blood	1x10^4 cp per mL

The PCR efficiency of LSI VetMAX™ African Swine Fever Virus detection kit, assessed from serial dilutions of a quantified ASF plasmid (pASF) until signal extinction, tested in triplicate, is close to 100% (Figure 3).

The limit of detection (LOD), evaluated on a quantified ASF plasmid, is estimated to be 16 copies of nucleic acids per PCR.

Repeatability and intermediate precision are evaluated at: coefficients of variation less than 4.09%.

For robustness, variations of temperature of hybridization (59° C, 60° C and 61° C), time of hybridization (54 sec, 60 sec and 66 sec), mix volume (18µL, 20µL and 22µL) and nucleic acid volume (4.5µL, 5µL and 5.5µL) do not affect the ASF PCR.

To determine the experimental limit of detection, serial dilutions of quantified plasmid are prepared to spike negative matrix at different concentration levels. The detection limit of MagMax™ Pathogen RNA/DNA method was estimated at 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood in individual samples.

Pooled assays are also performed by evaluating one positive sample among 5 or 10 samples. Serial dilutions of quantified plasmid are prepared to spike negative matrix as for individual tests. Then this positive sample is diluted in 4 or 9 negative samples to mimic pooled samples. The results of experimental LOD obtained show the same results as when tested individually (Table 4).

Table 4. Results obtained for the experimental limit of detection

	MagMAX™ Pathogen RNA/DNA Kit		
Matrices	**Individual analysis**	**Analysis of pool of 5**	**Analysis of pool of 10**
Serum	5x10^3 cp/mL	5x10^3 cp/mL	5x10^3 cp/mL
Blood	1x10^4 cp/mL	1x10^4 cp/mL	1x10^4 cp/mL

Pooled tests show the same experimental limits of detection (LOD) than individual tests. Furthermore this method enables to increase the analysis capacity in labs during outbreaks and reduce cost per analysis.

LSI VetMAX™ African Swine Fever Virus detection kit fulfills the validation criteria of PCR characteristics and complete method required by the NF U47-600-2 standard.

Table 5. Results obtained from field studies

5.1. Blood & Serum

Blood, Serum		Other methods		
		Positive	**Negative**	**Total**
LSI VetMAX™ African Swine Fever Virus detection kit	**Positive**	21	0	21
	Negative	0	1542	1542
	Total	21	1542	1563

The results show a correlation of 100% between both methods on positive blood and serum assays and show diagnostic specificity at: **Sp = 1542 / (1542+0) = 100%.**

5.2. Tissues

Tissues		Other methods		
		Positive	**Negative**	**Total**
LSI VetMAX™ African Swine Fever Virus detection kit	**Positive**	51	0	51
	Negative	0	6	6
	Total	51	6	57

The results show a correlation of 100% between both methods on negative tissues assays and show sensitivity at: **Se = 51 / (51+0) = 100%.**

For field studies, 1620 samples from various origins were tested. These field samples included various matrices (serum, blood and organs) at different levels of viral load (negative, low, medium, and high positive samples). 45 samples identified as positive or negative for ASF coming from a European Union Reference lab for ASFV (CISA-INIA, Valdeolmos, Spain); 1140 sera collected in a German slaughterhouse, a region free of ASFV; 400 blood samples collected on young pigs from weaning herds by a Spanish company, a region free for ASFV; and 36 samples from animal experiments carried out with 3 different ASFV strains from Central Veterinary Institute (CVI, Netherlands) were all evaluated.

Overall, in this assay, LSI VetMAX™ African Swine Fever Virus detection kit shows a diagnostic sensitivity of 100% on tissues and diagnostic specificity of 100% on blood and serum.

CONCLUSIONS

LSI VetMAX™ African Swine Fever Virus detection kit is a real-time PCR kit allowing the simultaneous detection of ASFV and an exogenous positive control in blood, serum and tissues samples.

The kit fulfills all the validation criteria for PCR characteristics and complete method required by the French standard (NF U47-600-2) "Requirements and recommandations for the development and validation of qRT-PCR in Animal Health".

The specificity, evaluated on different strains showed no cross-reactions with closely related pathogens. This kit had an efficiency close to 100% and its PCR limit of detection was16 copies per PCR (95% confidence interval).
The experimental LOD was 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood regardless of the test (individual or pool assays).
Test results on about 100 positive ASFV samples/strains and about 1600 negative samples showed 100% sensitivity on tissues and 100% specificity on blood and serum.

ASFV has significant economic impact and high mortality rate. The recent outbreaks of ASFV close to EU borders calls for a sensitive, reliable and specific real-time PCR such as the one described in this work.
LSI VetMAX™ African Swine Fever Virus detection kit provides a useful tool for an early detection of the ASF virus in various matrices from pigs and wild boars in order to guarantee the free status of pigs for trade. It helps enable control of the spread of disease and monitors circulating virus following outbreaks.

REFERENCES

▪ NF U 47-600-2 – Animal health analysis methods – PCR- Part 2: Requirements and recommandations for the development and the validation of veterinary PCR. (http://www.afnor.org)

ACKNOWLEDGEMENTS

▪ CISA-INIA, Valdeolmos, Spain
▪ CVI, Netherlands

TRADEMARKS/LICENSING

▪ LSI VetMAX™ African Swine Fever Virus detection kit (Cat. no. A28809)
▪ MagMax™ Pathogen RNA/DNA kit (Cat. No. 4462359)
▪ MagVet™ Universal Isolation kit (Cat. No. MV384)

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Certificate of Analysis

Certificat d'Analyse

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VetMAX™ African Swine Fever Virus Detection Kit

Σ	**100 Tests**	LOT	**ASFV-082**
REF	**A28809**		**2025-12-03**
			2026-11-27

KIT CONFIGURATION *CONFIGURATION DU KIT*

COMPONENT DESCRIPTION *Description du composant*	REF	UNIT	LOT	
3 - Mix ASFV	MPEASFV	2 x 1000 µL	MPEASFV-082	2026-12-02
4a - EPC ASFV	EPCASFV	2 x 90 µL	EPCASFV-079	2026-11-27
5 - IPC ASFV	IPCASFV	500 µL	IPCASFV-066	2026-11-27

INSTRUCTIONS FOR USE *Notices d'utilisation*

English	MAN0010783 RevD
Spanish	MAN0018204 RevC
French	MAN0019541 RevB

QUALITY CONTROL RESULTS *RESULTATS DE CONTROLE QUALITE*

EPC *EPC*	**Already extracted EPC** *EPC déjà extrait*	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
4a - EPC ASFV	$C_{t\,QC}$ ASFV = **24,8**	ABI 7500 (Applied Biosystems)

IPC *IPC*	**Extraction** - *Extraction :* **MagMAX™ CORE Nucleic Acid Purification Kit**	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
5 - IPC ASFV	$C_{t\,QC}$ IPC = **26,3**	ABI 7500 (Applied Biosystems)

$C_{t\,QC}$: Indicative Ct of the Quality Control *C_t indicatif du Contrôle Qualité*

SUPPLEMENTAL INSTRUCTIONS - Available upon request to Eurotech@thermofisher.com
Notices d'utilisation complémentaires - Disponibles sur demande auprès de Eurotech@thermofisher.com

Amplification Protocol *Protocole d'amplification*		**Nucleic acid purification Protocol** *Protocole de purification d'acides nucléiques*
Polish	MAN0018346 RevC	N/A

N. Fallery

Quality Assurance

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