

OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: VetMAX™ African Swine Fever Virus Detection Kit
Manufacturer: Thermo Fischer Scientific_ LSI S.A.S.

Disease: African Swine fever (ASF)

Pathogen Agent: ASF Virus P72 gene

Type of Assay: TaqMan® real-time PCR detection

Purpose of Assay: Fit for the purpose of detection of the African Swine Fever virus from blood, serum and tissues of pigs and wild pigs (including wild boars)

Species and Specimen: blood, serum and tissues of pigs and wild pigs (including wild boars)

1. Information on the kit

General information on the kit can be found on the Thermofischer website www.thermofischer.com

Tel: +33 (0)4.72.54.82.82

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2. Summary of validation studies

Analytical characteristics

Repeatability: PCR Repeatability is evaluated on three sessions by the same technician with the same material. Assays are performed with DNA of a quantified plasmid pASFV, diluted in TE 1X buffer to obtain 3 concentrations level (high/medium/low). Each sample is tested in triplicate. Repeatability of VetMAX™ African Swine Fever Virus Detection Kit shows coefficients of variation (CV) between 0.89 % and 3.01 %.

Analytical specificity: 100%

The analytical specificity of the kit was evaluated comparing PCR systems used in the kit (primers and probes) to ASFV sequences, present in National Center for Biotechnology Information public databases.

PCR inclusivity was evaluated on a panel of DNA extracted from 58 ASFV positive samples (organs, sera) from Centro de Investigacion en Sanidad Animal - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (CISA-INIA).

PCR exclusivity was evaluated on pathogens typically found in the same ecological niches, or phylogenetically close, or because they have the same clinical signs in the target species. VetMAX™ African Swine Fever Virus Detection Kit is specific for African Swine Fever Virus and does not detect other tested pathogens.

Analytical sensitivity: 100%.

The detection limit of the PCR (LDPCR) is the lowest concentration of target nucleic acid that will generate a positive result with a confidence of 95% (NF U47-600 standard). In order to determine the LDPCR experimentally, we should test, in terms of intra test (replica) and inter test (independent sessions), a range of target nucleic acid flanking the expected LDPCR value. The LDPCR was determined on a quantified plasmid pASFV to estimate the copy number of nucleic acids. Three individual dilution ranges were prepared by performing six 2-fold serial dilutions. The LDPCR is expected to fall within this range of dilutions.

The detection limit of VetMAX™ African Swine Fever Virus Detection Kit is 16 copies of nucleic acid per PCR.

From the results obtained throughout the analysis of experimental samples from animals with positive status, the CISA-INIA conclude that the VetMAX™ African Swine Fever Virus Detection Kit has appropriate analytical sensitivity and repeatability to give a confident ASF diagnosis based on the detection of ASFV genome.

Diagnostic Characteristics

Threshold Determination: The determination of the threshold consists of assigning a threshold cycle (C_t) value for each sample which depends on PCR design and thermal cycler used for the amplification. The threshold value is determined from the External Positive Control, in the middle of the exponential phase, in accordance with the NF U 47-600 standard:

Reaction type	ASFV Target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC} \text{ ASFV} \pm 3 C_t$	$C_t < 45$ or $C_t > 45$	PCR is validated
Extraction control	$C_t > 45$	$C_t = C_{tQC} \text{ IPC} \pm 3 C_t$	DNA extraction is validated
No - template control	$C_t > 45$	$C_t > 45$	PCR reagents are validated

IPC: Internal Positive Control

Interpretation of the Results

ASFV Target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	ASFV detected
$C_t > 45$	$C_t = C_t \text{ NEC} \pm 3 C_t$	ASFV not detected
$C_t > 45$	C_t is outside this range: $C_t \text{ NEC} \pm 3 C_t$	Invalid result

NEC: Negative Extraction Control

Diagnostic sensitivity (DSn) and specificity (DSp) estimates and 95% confidence intervals

VetMAX™ African Swine Fever Virus Detection Kit		Specimens
Diagnostic sensitivity	DSn = 100 %	51 tissue assays have been tested Se = 100% [93.02 – 100.0%]
Diagnostic specificity	DSp = 100%	1563 blood and serum assays have been tested Sp = 100% [99.76 – 100.0%] 63 tissue assays have been tested Sp = 100% [94.31 – 100.0%]

Comparative performance

The results obtained by CISA-INIA, in the analysis of the domestic pig and the European wild boar field samples obtained from ASFV genotype II infected animals in the Eastern European countries have been combined to provide an overall estimate of the performance of the kit for diagnosing ASF in field conditions. Out of the 424 samples, the number of positives using the UPL-PCR was 400 (94%) and 387 (91%) using the kit VetMAX™ African Swine Fever Virus Detection Kit.

Agreement and discrepancies

Concerning the studies performed by CISA-INIA:

- From the analysis of 404 field samples obtained from epidemic areas in eastern Europe, the kappa value was 0.87 indicating near perfect agreement between the UPL reference method and VetMAX™ African Swine Fever Virus Detection Kit.
- From the analysis of 16 EURL ASF Reference panel, there was perfect agreement between the UPL reference method and VetMAX™ African Swine Fever Virus Detection Kit.
- Among the UPL reference method and VetMAX™ African Swine Fever Virus Detection Kit there was perfect agreement in the analysis of 136 experimental blood samples tested, with Ct values lower than 30.
- From the analysis of tissue samples, the ASFV genome was detected by the UPL-PCR and by the VetMAX™ PCR kit in 100% of tested tissues showing a 100% agreement among both methods.

Reproducibility

The robustness has been evaluated by checking the ability of the PCR run to remain unaffected by variations in critical parameters of a PCR reaction:

Test 1: T°C of hybridization +/- 1°C

Test 2: Time of hybridization +/- 10 %

Test 3: Volume of PCR mix +/- 10 %

Test 4: Volume of DNA +/- 10 %

VetMAX™ African Swine Fever Virus Detection Kit successfully passed the robustness challenge.

The testing of a unique panel of 15 biological samples (11 positive and 4 negative samples; characterized with CISA-INIA method; panel of 15 reference samples, inactivated and lyophilized) by both Thermo Fischer laboratory and CISA-INIA Valdeolmos with VetMAX™ African Swine Fever Virus Detection kit provided the following results: the Ct values obtained for the 15 samples showed coefficients of variation (CV) between 2.75 and 7.20%.

The kit provided consistent qualitative results, and the results were not affected by environmental factors.

Applications

The VetMAX™ African Swine Fever Virus Detection Kit is used for diagnosis of African Swine fever virus (ASFV).

For Veterinary Use Only and for in Vitro Use Only.

References

Tignon M. *et al.* (2011). Development and inter-laboratory validation of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African Swine Fever virus. *Journal of Virological Methods*, **178** (1-2): 161-170.

Gallardo C. *et al.* (2015). Assessment of African Swine Fever diagnostic techniques as a response to the epidemic outbreaks in Eastern European Union countries: How to improve surveillance and control programs. *Journal of Clinical Microbiology*, **53**(8): 2555-2565.

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