



WOAH Procedure for Registration of Diagnostic Kits  
Validation Studies Abstract

Name of the diagnostic kit: Genelix™ ASFV Real-time PCR Detection kit

Manufacturer: Sanigen Co., Ltd.

Procedure /Approval number: 052131

Date of Registration: May 2024

Disease: African Swine Fever (ASF)

Pathogen Agent: ASF Virus

Type of Assay: Real-time PCR

### Purpose of Assay

The Genelix™ ASFV Real-time PCR Detection kit is a product that qualitatively detects and confirms the diagnosis of ASFV using a real-time PCR detection system in the whole blood, serum, and tissues of swine suspected of being infected with the ASFV.

### Species and Specimens

The target species is domestic swine, and samples of whole blood, serum, and tissues can be used for testing. Whole blood stored with anticoagulants can be used for the tests. It is recommended that specimens should be tested as soon as possible after collection. However, if immediate use is not achievable, the specimens can be stored for a few days at 4 °C in a fridge or more than seven days at colder than -70 °C in a deep freezer. Specimens should be divided into amounts required for tests and stored at -20±5 °C in a freezer to avoid thawing repeatedly. If the processing or transport is delayed more than 24 hours, it should be kept at -20±5 °C. Avoid repeated freeze and thaw.

#### 1. Information on the kit

Please refer to the kit insert available on the WOAH Registry web page or contact the manufacturer at Sanigen Co., Ltd.

Tel: +82-1833-8010

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#### 2. Summary of validation studies

##### Analytical specificity

**Conclusion:** The interference reaction tests using the positive and the negative samples with five types of interfering substances indicate no interference with results. The cross-reactivity test was evaluated to distinguish between the target and non-target analytes. Exclusivity was confirmed with pathogens related to swine disease or infectious reagents (41 materials, including 16 bacteria, seven swine disease related viruses, and 18 other viruses). No significant cross-reactivities were found. Nine genotypes of the ASFV p72 gene, the target analyte for the

kit, were synthesized and tested for inclusivity. As a result, all types of the gene were detected as positive.

### Analytical sensitivity

**Conclusion:** The limit of detection (LOD) test of the Genelix™ ASFV Real-time PCR detection kit was performed to measure analytical sensitivity. The significant low positive concentrations were repeated 24 times, and the data were reanalyzed using probit analysis in 95 % confidence; as a result, the maximum estimate of 16.9 ( $1.7 \times 10^1$ ) copies/ $\mu\text{l}$  was reported as the LOD.

### Repeatability

**Conclusion:** The repeatability was conducted with one person, one lot, for 20 days, with two runs per day, duplication per run, and three different concentrations. As a result of experiments by diluting the ASFV plasmid DNA to the three levels of sample concentration, 100 % of all samples were detected, and negative control showed no amplification in all samples. The coefficient of variation (CV) value was less than 5 % in all cases.

### Diagnostic characteristics:

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

#### **Conclusion:**

**Threshold determination:** The threshold (cut-off) of the Genelix™ ASFV Real-time PCR detection kit is 38.1 Ct. For LOD determined by probit analysis, the cut-off is defined as the average Ct value of the next most concentrated dilution tested to the LOD defined by probit. In the cut-off evaluation, the average Ct value was 38.1 at  $2.8 \times 10^1$  copies/ $\mu\text{l}$ , which is the closest concentration above the probit value.

### Interpretation of the result

The criteria for setting threshold and baseline according to the equipment are as follows.

Instrument	Threshold	Baseline start	Baseline end
AB 7500	0.1	3	15
AB 7500 Fast	0.1	3	15
QuantStudio™ 5	0.4	3	15
Bio-rad CFX96™	100	3	15

If the positive and negative control results match the following criteria listed in the table, interpret the results for the target sample(s). If the results of the control materials do not match the table, repeat the experiment.

Control type	Ct value
Positive Control	$Ct \leq 38.1$
Negative Control	Non-Detected

Check the Ct value of the sample(s) using the instrument-specific software. The sample data is considered positive at  $Ct \leq 38.1$  and negative at  $Ct > 38.1$ .

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

To evaluate the diagnostic sensitivity and specificity, a comparative test was conducted using the reference method (validated and certified by the WOA), and the results are depicted below.

Genelix™ ASFV Real-time PCR detection kit		ASFV/Swine Whole blood & Serum
Diagnostic sensitivity	N	187
	DSe	99.47 %
	CI	97.07 to 99.99%
Diagnostic specificity	N	553
	DSp	100 %
	CI	99.33 to 100.0%

Genelix™ ASFV Real-time PCR detection kit		ASFV/Swine Tissue
Diagnostic sensitivity	N	22
	DSe	100 %
	CI	84.56 to 100.0%
Diagnostic specificity	N	450
	DSp	100 %
	CI	99.18 to 100.0%

## Reproducibility

**Conclusion:** Three WOA reference laboratories for ASF conducted a comparison study on reproducibility. For the reproducibility of the test, three labs, three days, and two runs per day were compared. All qualitative results were 100% in agreement and met the acceptance criteria with less than CV 5 %. The test results are shown in the table below:

Sample No.	Coefficients of Variation (%)			
	Sanigen	Lab A	Lab B	Lab C
SNG-01	1.09	0.46	0.90	1.36
SNG-02	0.68	2.81	0.43	1.19
SNG-03	0.40	0.40	0.40	2.42
SNG-04	0.68	0.92	2.56	1.66
SNG-05	2.20	2.86	1.86	2.28
SNG-06	Negative	Negative	Negative	Negative
SNG-07	Negative	Negative	Negative	Negative
SNG-08	0.87	2.36	1.64	0.92
SNG-09	0.21	4.98	2.07	0.45
SNG-10	0.63	1.66	1.29	0.64
SNG-11	0.60	0.57	0.62	1.29
SNG-12	0.90	1.55	0.95	0.33
SNG-13	0.19	2.12	0.47	0.69
SNG-14	0.36	0.91	0.92	1.41
SNG-15	Negative	Negative	Negative	Negative
SNG-16	0.60	5.18	1.07	0.78
SNG-17	1.04	0.42	0.43	1.00
SNG-18	1.03	2.07	1.02	1.08

SNG-19	1.02	6.13	1.54	1.71
SNG-20	Negative	Negative	Negative	Negative

## Reference

1. Chapter 1.01.06 Principles and Methods of validation of diagnostic assays for infectious diseases (WOAH 2023)
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7. Chapter 2.2.6 Selection and use of reference samples and panels (WOAH 2024)
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9. CLSI-EP07-A2 Interference Testing in Clinical Chemistry
10. CLSI-EP05-A3 Evaluation of Precision of Quantitative Measurement
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13. Addressing African swine fever (FAO, 2020)