World Organisation for Animal Health
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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:

Date of Registration:

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 Genelix™ ASFV Real-time PCR Detection kit is a product that qualitatively detects and confirms the diagnosis of ASFV in Swine's whole blood, serum, and tissues. Specimens stored with anticoagulants can be used for the tests. It is recommended that specimens shall be used immediately after collection. However, if immediate use is not achievable, the specimens can be stored for a few days at 4 ℃ in a fridge or more than seven days at colder than -70 ℃ in a deep freezer. Specimens should be divided into amounts required for tests and stored at -20±5 ℃ 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 ℃. 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 manufacturer at: Sanigen Co., Ltd.
Tel: +82-1833-8010
Fax: +82-2-573-3134

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 relative viruses, and 18 other viruses). There were no significant cross-reactivities found. Nine genotypes of the ASFV p72 gene 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 x 101) copies/□ 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 test, the average Ct value is 38.1 at 2.8 x 101 copies/µl, 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, set the experiment again

Control type	Ct value
Positive Control	Ct ≤ 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 ≤ 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 WOAH), and the results are depicted below.

Genelix™ ASFV Real-time PCR detection kit		**ASFV/Swine Whole blood & Serum**
Diagnostic sensitivity	N DSe CI	190 99.47 % 97.07 to 99.99%
Diagnostic specificity	N DSp CI	552 100 % 99.33 to 100.0%

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

Reproducibility

Conclusion: The selected three WOAH reference laboratories 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;

	Coefficients of Variation (%)			
Sample No.	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)
2. Chapter 2.02.03 Development and optimisation of nucleic acid detection assays (WOAH 2024)
3. Section 3.8-SUIDAE Chapter 3.8.1-African Swine Fever (Infection with African swine fever virus) (OIE 2019)
4. African Swine Fever Virus: A Review. Viruses 2017, 9, 103; doi: 10.3390/v9050103
5. African swine fever: detection and diagnosis. A manual for veterinarians. Food and Agriculture Organization of the United Nations. 2017
6. Chapter 1.01.02 Collection, submission and storage of diagnostic specimens (WOAH 2018)
7. Chapter 2.2.6 Selection and use of reference samples and panels (WOAH 2024)
8. CLSI-EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
9. CLSI-EP07-A2 Interference Testing in Clinical Chemistry

10. CLSI-EP05-A3 Evaluation of Precision of Quantitative Measurement
11. King, D. P., S. M. Reid, G. H. Hutchings, S. S. Grierson, P. J. Wilkinson, L. K. Dixon, A. D. S. Bastos, and T. W. Drew, 2003: Development of a TaqMan® PCR assay with internal amplification control for the detection of African swine fever virus. J. Virol. Methods 107, 53–61
12. Charles G.B. Caraguel, 1 Henrik Stryhn, Nellie Gagne', Ian R. Dohoo, K. Larry Hammell, Selection of a cutoff value for real-time polymerase chain reaction results to fit a diagnostic purpose: analytical and epidemiologic approaches
13. Addressing African swine fever (FAO, 2020)