World Organisation for Animal Health

WOAH Procedure for Registration of Diagnostic Kits
Validation Studies Abstract

Name of the diagnostic kit: Sentinel® ASFV Antibody Rapid Test

Manufacturer: Excelsior Bio-System Incorporation

Procedure /Approval number: 062233

Date of Registration: May 2024

Disease: African swine fever

Pathogen Agent: African swine fever virus

Type of Assay: Immuno-chromatographic lateral flow assay (Rapid test)

Purpose of Assay: Detection of antibody associated with current infection or an immune response to previous exposure in an individual animal, group of animals or defined population. For use in conjunction with other tests or diagnostic procedures, as an aid in diagnosis or other clinical or epidemiological assessments.

Species and Specimens: Porcine serum

1. Information on the kit

Please refer to the kit insert available on the WOAH Registry web page or contact manufacturer at:
Website link: ebs.com.tw/en/products/asfvrt
Email address: sales@ebs.com.tw

2. Summary of validation studies

Analytical specificity

Conclusion:

a) Sentinel® ASFV Antibody rapid test can be used for serum sample from different genotypes (I, II, IX, X) of African swine fever virus infection.

b) Sentinel® ASFV Antibody Rapid Test can provide a high-specificity result (93/95 = 97.89%; 95% CI = 92.6% to 99.74%) with a very low cross-reactivity for 95 individual samples from 19 typical pig pathogens (non ASFV) of the domestic pigs.

c) Potential interfering factors, such as anticoagulants, hemolysis (hemoglobin) and lipaemia (intralipid), did not affect the test results.

Analytical sensitivity

Conclusion

There was more than 80% agreement between the EURL-IPT test and Sentinel test when the sera had antibody titers higher than 1:5120.

Repeatability

Conclusion

For the intra-assay, an operator evaluated 4 reference sera (strong, medium, weak, and negative) in quadruplicate tests. Inter-assay agreement was evaluated using the same 4 reference sera in 20 runs by three operators on separate days with different batches of kits. All intra-assay and inter-assay runs of the four reference sera produced identical results. The Sentinel® ASFV Antibody Rapid Test demonstrated 100% repeatability. According to the European Reference Laboratory (EURL) intra-assay and inter-assay reports, 10 reference sera were tested in one round/day for 2 days, and each round was tested in duplicate. The Sentinel® ASFV Antibody Rapid Test had 100% repeatability.

Diagnostic characteristics

Threshold determination

Sentinel® ASFV Antibody Rapid Test is a qualitative test. The test sample is positive when two lines (C line and T line both) appear and negative when only the C line appears. The threshold (cut-off) of antibody titer is > 1:640 (> 50% agreement with EURL-IPT test).

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

788 serum samples have been tested. The results obtained from EURL and Excelsior Bio-System evaluation report.

	EURL-IPT		ASFV free
	Positive	Negative	Negative
Category 1: EURL-ASF-Ref1	8	2	-
Category 2: Reference experimental serum	122	23	-
Category 3: Experimental samples from pigs infected with genotype II ASFV	148	96	-
Negative serum samples from National Pingtung University of Science and Technology (NPUST), Taiwan	-	-	389
Total	278	121	389

Sentinel® ASFV Antibody Rapid Test		Specimens

Diagnostic Sensitivity (DSe)	**81.65%** (95% CI = 76.60% to 86.02%)	EURL-IPT Positive: 278
Diagnostic Specificity (DSp)	**96.27%** (95% CI = 94.24% to 97.74%)	EURL-IPT Negative:121 NPUST ASFV Free: 389

Reproducibility

Conclusion:

The reproducibility study was performed by the Pirbright Institute and evaluated in three laboratories. 22 positive and 20 negative samples, as determined by ELISA (the reference standard), were tested. The results indicate the Sentinel® ASFV Ab Rapid Test can produce results with a reasonable degree of reproducibility when used to test replicate samples in different laboratories. The kappa values of interlaboratory comparison are following.

Interlaboratory	Kappa Value	Result
Lab 1 and Lab 2	**0.781** (95%CI = 0.582 to 0.981)	substantial agreement
Lab 1 and Lab 3	**0.850** (95%CI = 0.695 to 1.000)	very high agreement
Lab 2 and Lab 3	**0.791** (95%CI = 0.603 to 0.979)	substantial agreement

References

1. Afonso CL, Alcaraz C, Brun A, Sussman MD, Onisk DV, Escribano JM, Rock DL. Characterization of p30, a highly antigenic membrane and secreted protein of African swine fever virus. Virology. 1992 Jul;189(1):368-73.

2. Giménez-Lirola LG, Mur L, Rivera B, Mogler M, Sun Y, Lizano S, Goodell C, Harris DL, Rowland RR, Gallardo C, Sánchez-Vizcaíno JM, Zimmerman J. Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA. PLoS One. 2016 Sep 9;11(9):e0161230.

3. Gallardo C, Fernández-Pinero J, Arias M. African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. Virus Res. 2019 Oct 2;271:197676.