# Annex 19. 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**:

**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

1. **Summary of validation studies**

**Analytical specificity**

***Conclusion*:**

1. Sentinel® ASFV Antibody rapid test can be used for serum sample from different genotypes (I, II, IX, X) of African swine fever virus infection.
2. 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.
3. Potential interfering factors, such as anticoagulants, haemolysis (haemoglobin) 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 titres 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 titre 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:121NPUST 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 C.L., Alcaraz C., Brun A., Sussman M.D., Onisk D.V., Escribano J.M. & Rock D.L. (1992). Characterization of p30, a highly antigenic membrane and secreted protein of African swine fever virus. *Virology*, **189**, 368–373.

2. Giménez-Lirola L.G., Mur L., Rivera B., Mogler M., Sun Y., Lizano S., Goodell C., Harris D.L., Rowland R.R., Gallardo C., Sánchez-Vizcaíno J.M. & Zimmerman J. (2016). 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,* 11(9):e0161230.

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