Sentinel® ASFV Antibody Rapid Test

Cat. No. 4333007

World Organisation for Animal Health

For veterinary use only.

Intended Use

The validation data for this kit have been certified by WOAH, based on expert review, as fit for the following purposes:

Sentinel® ASFV Antibody Rapid Test is a qualitative immunochromatographic assay for the detection of antibodies against African swine fever virus (ASFV) in porcine serum. 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.

Introduction

The outbreak of African swine fever (ASF) poses a serious threat and losses to the swine industry worldwide. Because no effective vaccine is available, the presence of antibodies against ASFV is used as an indicator of infection. Therefore, detection of the antibodies against ASFV is important on ASF surveillance and Sentinel® ASFV Antibody Rapid test could be a convenient tool for laboratory and field office applying to prevent ASF spreading.

Principles of Procedure

Sentinel® ASFV Antibody Rapid Test is a lateral flow immunoassay based on the use of colloidal gold. The colloidal gold was designed to bind the antibody in serum or plasma specimen. The recombinant viral protein p30 which is the most antigenic protein of ASFV and thus could be appropriate antigen for antibodies detection was used as test line to capture reagents. Once there is anti-ASFV p30 protein antibodies in porcine sample, the antibodies will be captured on the test line.

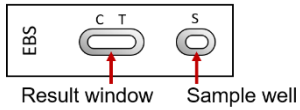
Package Components

30 aluminum foil pouches each containing one ASFV Antibody test card, one disposable capillary tube, and one desiccant.

1	ASFV antibody test device (Figure 1.)	30
2	Disposable capillary tubes (Figure 2.)	30
3	Desiccant	30
4	Running buffer	1 x 5 mL
5	Package insert	1

Note: This kit does not contain infectious material.

Figure 1.



The sample well marked “S” and the result window marked “C” and “T” on the test card.

Figure 2.

The sample could be collected by capillary action and that 10 µL of sample can be dispensed into the sample well by depressing the bulb of disposable capillary tube.

Materials Required but Not Provided

- Digital timer

Sample Requirement

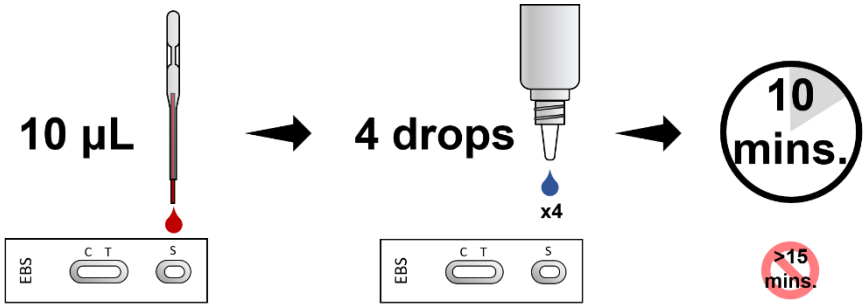
The fresh, refrigerated (less than 5 days at 2-8 °C) and frozen serum samples from pig can be used for this test.

Test Procedure

Bring all reagents to room temperature 15-30°C before use.

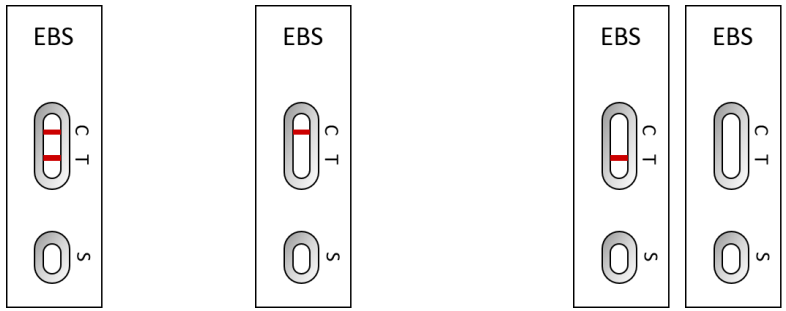
Check the package components before testing. If the desiccant color is pink, this test card is recommended not to be used.

1. Add **10 µL sample** collected by disposable capillary tubes into the sample well.
2. Hold dropper bottle straight over the sample well, not at an angle.
3. Put **4 drops running buffer** into the sample well sequentially.
4. Interpret test results at **10 minutes**. Do not interpret after 15 minutes.



Interpretation of Results

- Positive:
  Appearing two distinct pink/red line on the control (C) line and test (T) line indicate the presence of the antibodies against ASFV in the sample.
- Negative:
  Appearing only one distinct pink/red line on the control line (C) indicates the absence of the antibodies against ASFV in the sample.
- Invalid:
  Control (C) line does not appear, the result be considered invalid. The sample should be retested with a new device.
- Inconclusive:
  If there are inconclusive test results (faint line) in test (T) line, it may be in early immune response. The inconclusive result suggests retesting or resampling.



Positive Negative Invalid

Storage

The kit should be stored at 2-30°C until expiration date marked on the label.

Summary of the validation data

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), Chinese Taiepi	-	-	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 Antibody 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

Precautions

- The test procedure must be strictly followed.
- Handle the specimens and materials contacting sample as potentially infectious.
- Wear protective clothing such as laboratory coats, disposable gloves and eye or face protection when handling sample and reagents.
- Do not use the test kit beyond the expiration date marked on the label.
- Do not mix components from different lot numbers.
- Do not use the test card if the aluminum foil pouch is damaged or unsealed.
- Follow the national and local regulations to decontaminate and dispose of all samples, used components and potentially contaminated materials safely.

References

- OIE Terrestrial Manual 2012, Chapter 2.8.1, African swine fever.
- African Swine Fever Gap Analysis Report 2018, Global African Swine Fever Research Alliance.
- Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins. J Virol. 2018 Mar 28;92(8).
- 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 Jul 2015, 53 (8) 2555-2565; DOI: 10.1128/JCM.00857-15

Excelsior Bio-System Incorporation

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