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Sentinel

QA about African Swine Fever

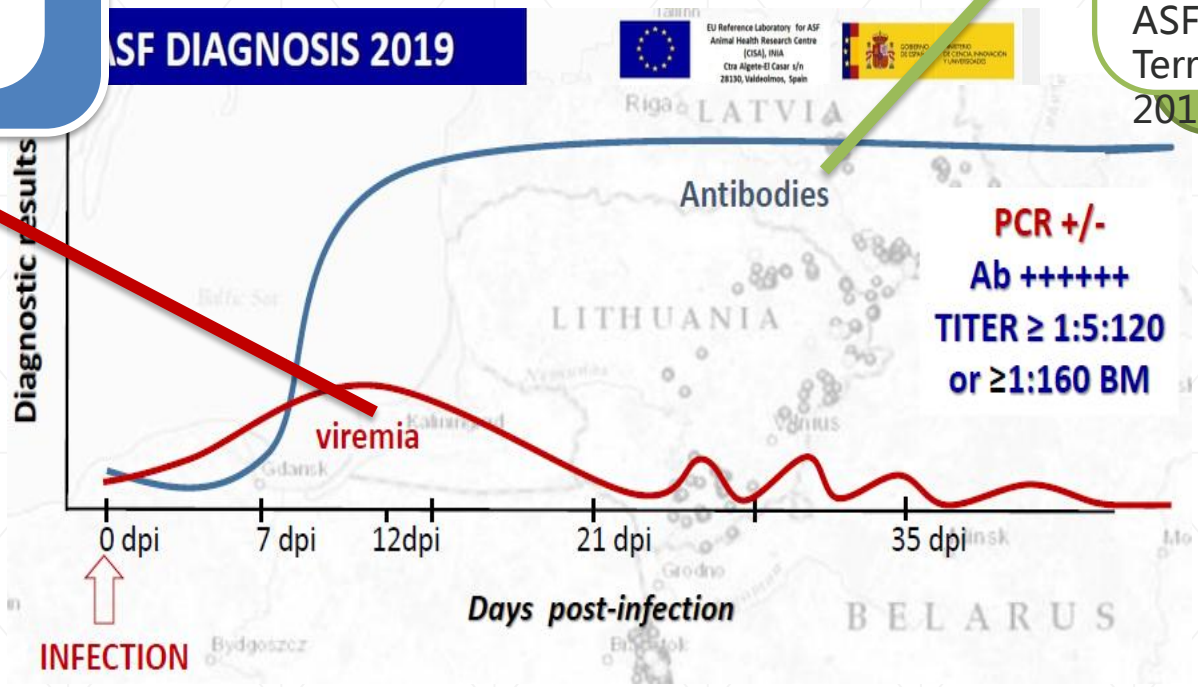
Sentinel® African Swine Fever Virus Antibody Rapid Test

2020/05/11

Q : How soon will ASF virus or antibody appear after the infection?

Rising rapidly 4-8 days after infection and then decline with time. For pigs infected more than 35 days, viral nucleic acid has not been detected.

Appears 7-10 days after infection and lasts for life (up to 5 years). An excellent diagnostic tool especially in subacute and chronic ASF infection. (OIE Terrestrial Manual 2019)



Diagnostics in the Veterinary Field : The Role in Health Surveillance and Disease Identification THURSDAY 16 May , 2019, OIE Terrestrial Manual 2019.

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Sentinel® African Swine Fever Virus Antibody Rapid Test

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Sentinel® African Swine Fever Virus Antibody Rapid Test content ?

- Each box contains: 30 bags, 1 paper of instruction and 1 bottle of buffer
- Each bag contains: 1 rapid test, 10 µL quantitative pipette, 1 desiccant

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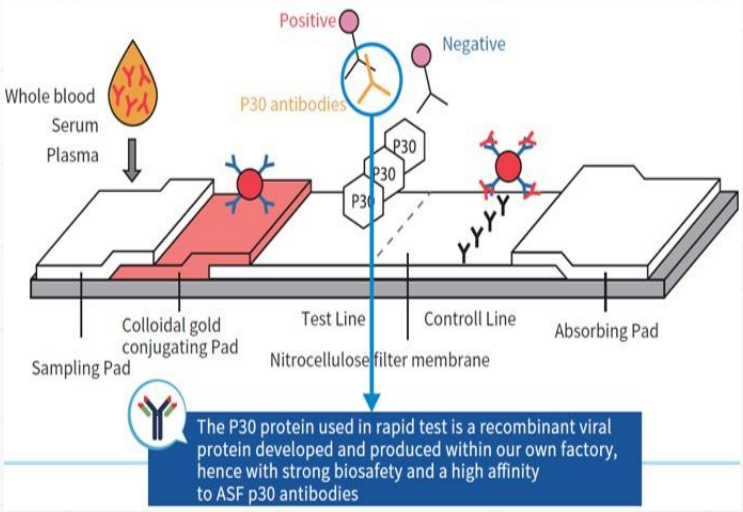
A brief introduction about Sentinel® African Swine Fever Virus Antibody Rapid Test

Sentinel® African Swine Fever Virus Antibody Rapid Test uses lateral flow immunochromatographic methods combined with nano-colloid gold particles. When blood sample is added, ASFV-induced antibody in the sample will first bind to nano-colloidal gold particles, then antigen-antibody reaction will happen on the T line expressed viral protein 30 (Figure 1). If sample contains enough viral P30 antibody, the nano-colloidal gold particles will show a red signal on the T line, which is regarded as positive result; if the sample does not contain the viral P30 antibody, the detection on T line will not show any color , regarded as negative result (Picture 1).



(Figure 1) lateral flow immunochromatographic methods

(Picture 1) The left side show 3 positive results for Sentinel® African Swine Fever Virus Antibody Rapid Test; and the right side show 3 negative results.

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Q : Why is the detection of P30 antibody better than P72?

- A : In the research, we found that serological method of ELISA became the most commonly used diagnostic test due to its simplicity and relatively low cost. However, be careful when using P72 for the serological diagnosis, because the P72 gene is highly variable (not conservative) especially at the end of the antibody (Boshoff et al., 2007; Michaud et al., 2013; Muangkram et al., 2015; Achenbach et al., 2017; Quembo et al., 2018). By comparing P30 gene sequence from 86 ASFV isolates, we found that 3 of the 4 linear epitopes of P30 antibody are highly conservative. Serological methods of conserved epitopes can provide a wider range of diagnosis.
- P30 is the surface protein of ASFV and can be detected from **2 to 4 days post infection**. The protein is produced before the viral DNA synthesis and keeps expressing until the end of the virus life cycle (Afonso et al., 1992 ; Prados et al., 1993). It is immunogenic and can stimulate the highest level of antibody response during ASF infection (Giménez-Lirola et al., 2016).

(来源：Antigenic Regions of African Swine Fever Virus Phosphoprotein P30. Ping Wu1, Andre D. Lowe1, Yelitza Y. Rodríguez, Maria V. Murgia, Kimberly A. Dodd, Raymond R. Rowland, Wei Jia.)

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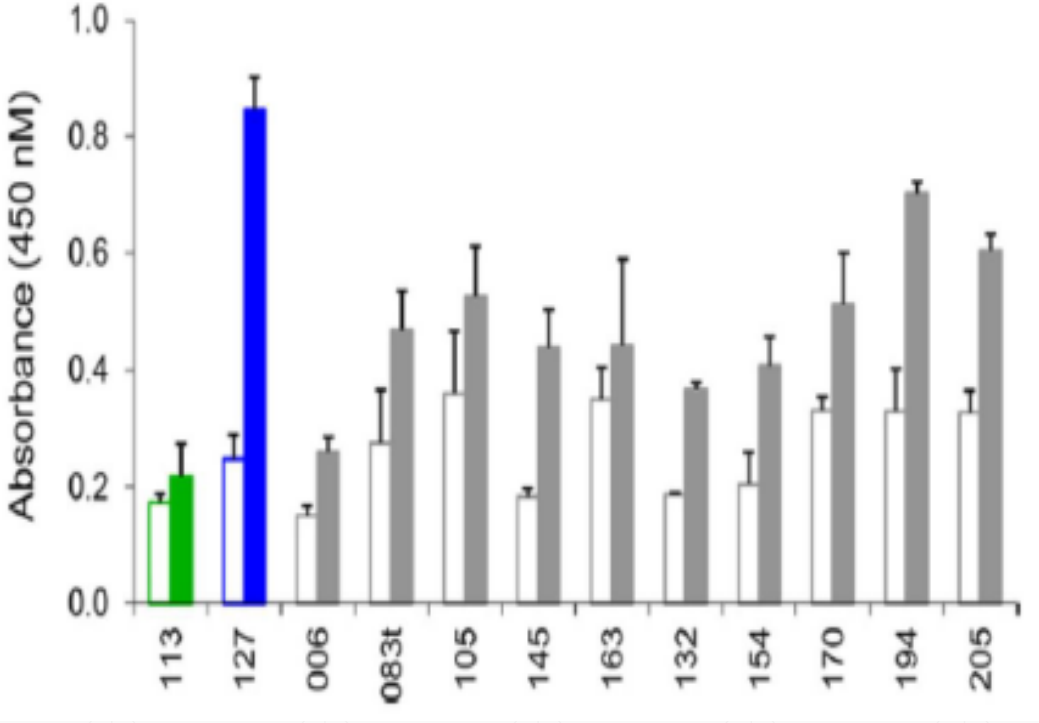
Q : Why is the detection of P30 antibody better than P72 ?

- Jancovich et al. Found that P30 stimulated the cellular and humoral immunity to the greatest extent among many ASFV antigens (Jancovich et al., 2018). Using ELISA to detect the reactivity of pig sera before and after immunized by different antigens, results showed that P30 serum (127, the blue bar) had the largest difference in absorbance value, reaching 3-4 times, and the OD was the highest, which was easy to recognize (Figure) (2018).



Reactivity of pig serum to every ASFV antigens before and after immunization

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Q : Why is the detection of P30 antibody better than P72 ?

- The fluorescence intensity of antibodies against P30 in serum is significantly higher than that of P54 and P72, indicating that **this antigen is the best candidate for serum detection**.

- Previous reports indicate that ASFV proteins 30, 54 and 72 may be the diagnostic antigens. The results of the experimental samples show that P30 provides the best diagnostic performance, which **can detect induced antibodies at an early stage after ASFV exposure**.

Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA. Luis G. Giménez-Lirola1*, Lina Mur2, Belen Rivera2, Mark Mogler3, Yaxuan Sun4, Sergio Lizano5, Christa Goodell5, D. L. Hank Harris3, Raymond R. R. Rowland6, Carmina Gallardo7, José Manuel Sánchez-Vizcaíno2, Jeff Zimmerman1

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Q : Sentinel® Advantages : 4

1. Nano-colloidal gold particles, accuracy up.
2. Lock on P30 to detect earlier stage in antibody response.
3. Less sample volume and easy SOP.
4. Validated by OIE and EU.

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Q : Sentinel® Advantages-1 ?

- A : Nano-particle level enables Sentinel® combine more antibodies in the blood than common latex particle, showing more obvious coloring and reducing any false judgment.

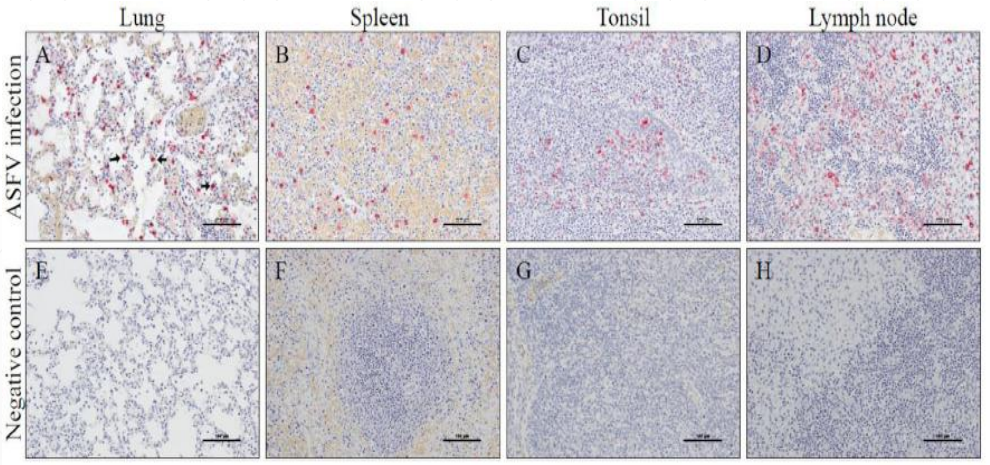
Serum in ASFV free areas

Sentinel®
P30

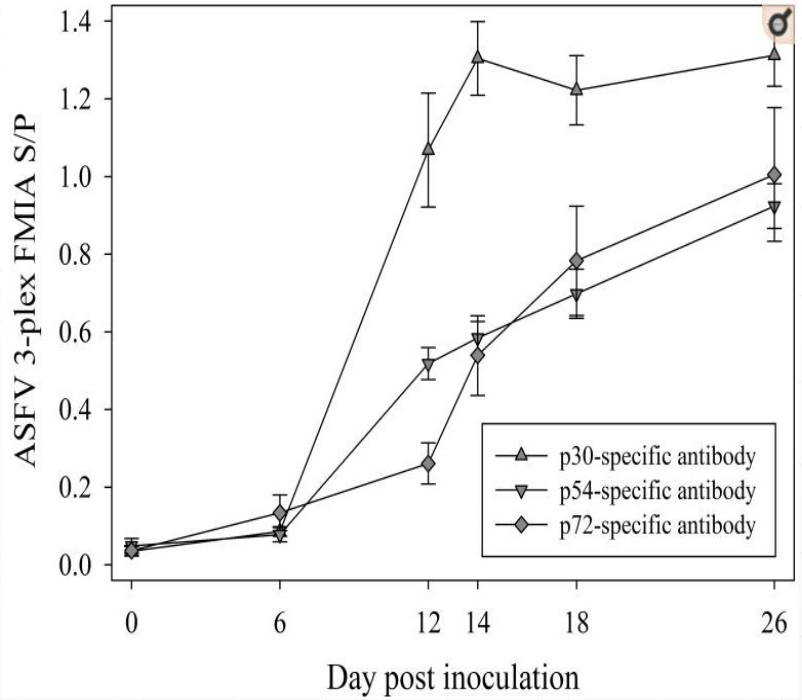
Other brand
P72

Q : Sentinel® Advantages-2 ?

- A : (1) P30 is one of the most immunogenic proteins, and (2) is produced in ASFV early infection stage. These two characteristics make P30 a excellent target for detecting ASFV.



(Figure 1) Using Immunohistochemistry (IHC) against viral P30 to detect ASFV-infected tissues, it can be found that P30 is abundant in each infected tissue.



(Figure 2) The ratio of fluorescence each days after fluorescent labeling on common ASF viral proteins. P30 has risen sharply on the 6th day of infection (one week later) and over P72 both the value or the slope, showing that P30 can be used as one of the perfect

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Q： Sentinel® Advantages-3 , three stpes !

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Q： Sentinel® Advantages-4, An international pride!!! Validated by OIE and EU reference lab.

西班牙

Center for Veterinary Health Surveillance (VISAVET)

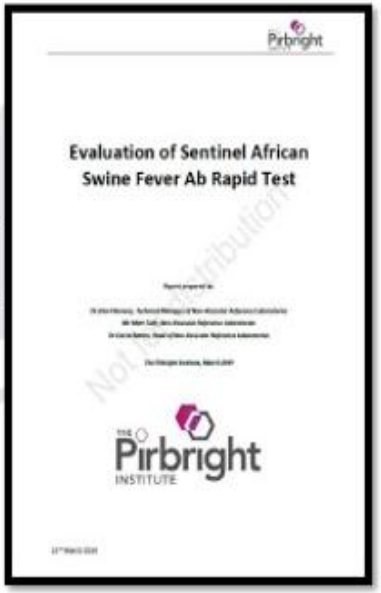
European Reference Lab for ASF


Evaluation of the Sentinel ® ASFV Ab Rapid Test

ASSESMENT REPORT

英國

The Pirbright Institute


Evaluation of Sentinel African Swine Fever Ab Rapid Test

Pirbright INSTITUTE

南非

Agricultural Research Council Onderstepoort Veterinary Institute

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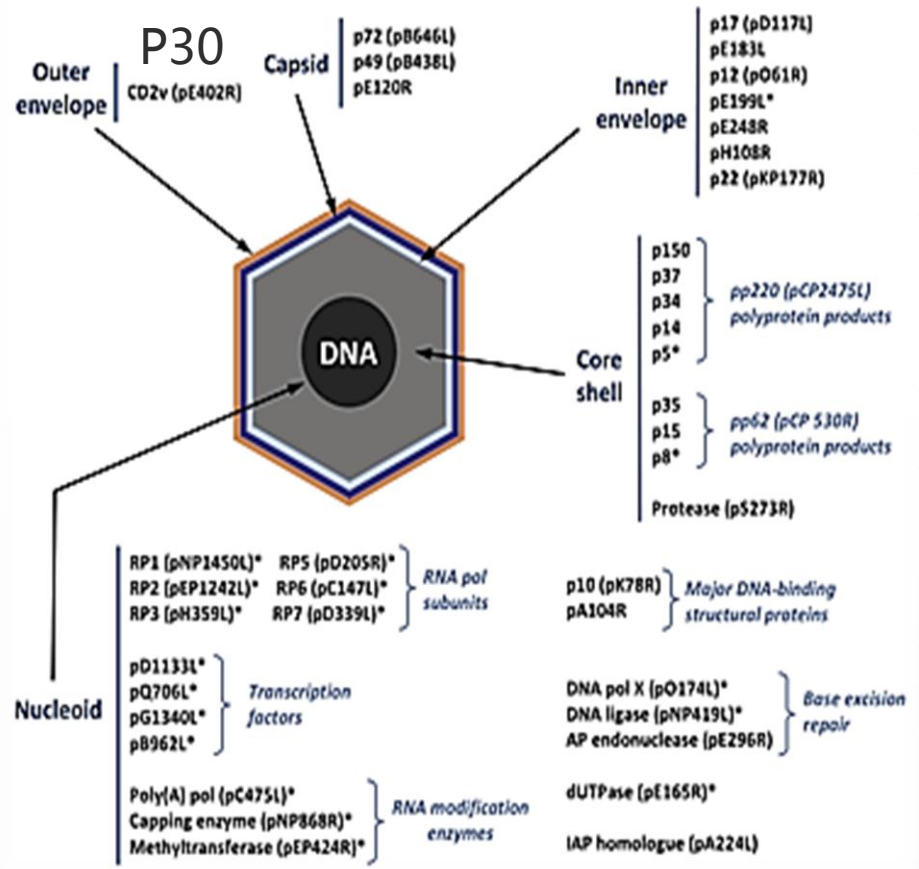
Q : Which genotype of ASFV can be detected ?

- A：Due to the serological method, Sentinel® African Swine Fever Virus Antibody Rapid Test can detect ASFV infections of genotype 1 (previous invasion in Europe) and genotype 2 (currently in Asia) ！

Q : Whether other swine diseases affect the test result ?

- A：No. This product is against specific viral protein 30 on the surface of ASFV, so these antibodies in the blood do not have the affinity to bind proteins of other swine disease virus.



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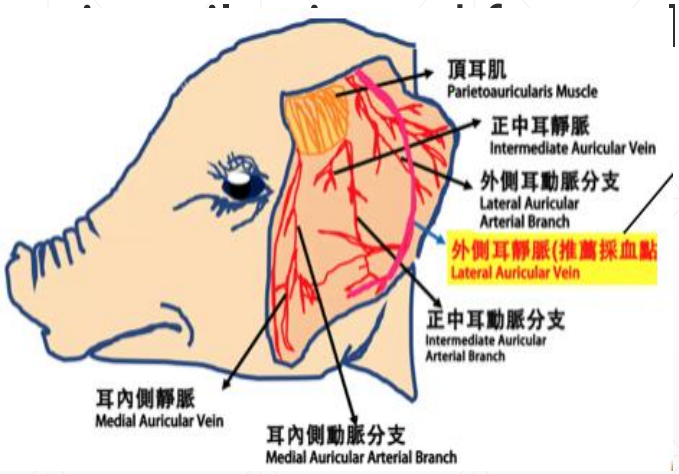
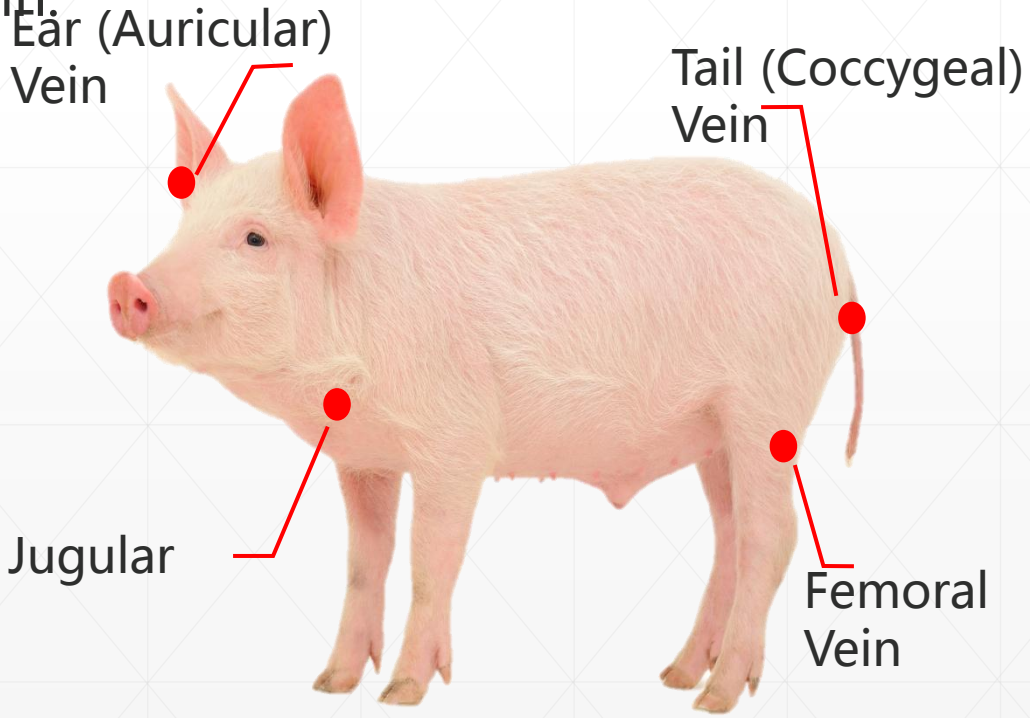
Instructions

Q : Step 1 2 3, what is the first step?

- A：Blood must be sampled first. Ear vein is often used as a blood sampling point, because the wound is small and easy to disinfect.
- Another common blood sampling points are external jugular vein.





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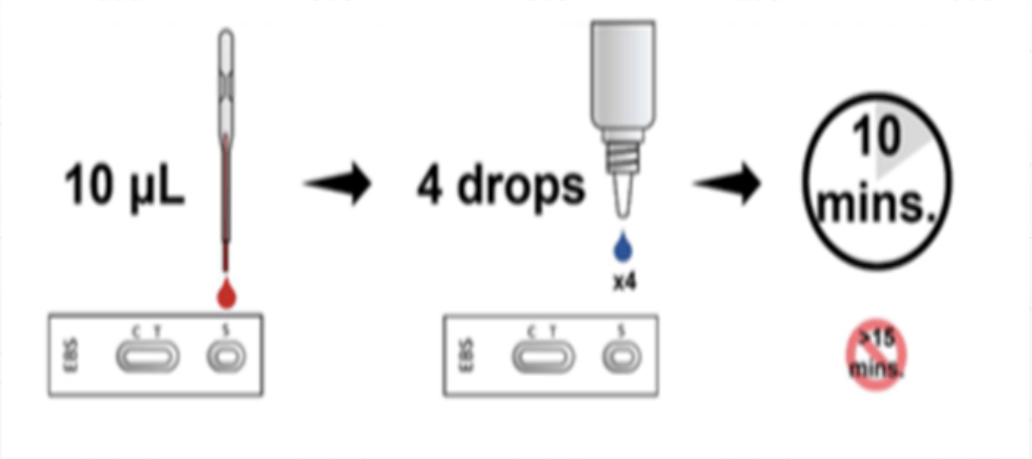
Q : Step 1 2 3, and the second step?

- A : Using the attached quantitative pipette, draw the whole blood / serum / plasma till the marked scale (10 µL) and drip it into the sample well, then add 4 drops of buffer solution, wait for 10 minutes, the result will show up, do not exceed 15 minutes.



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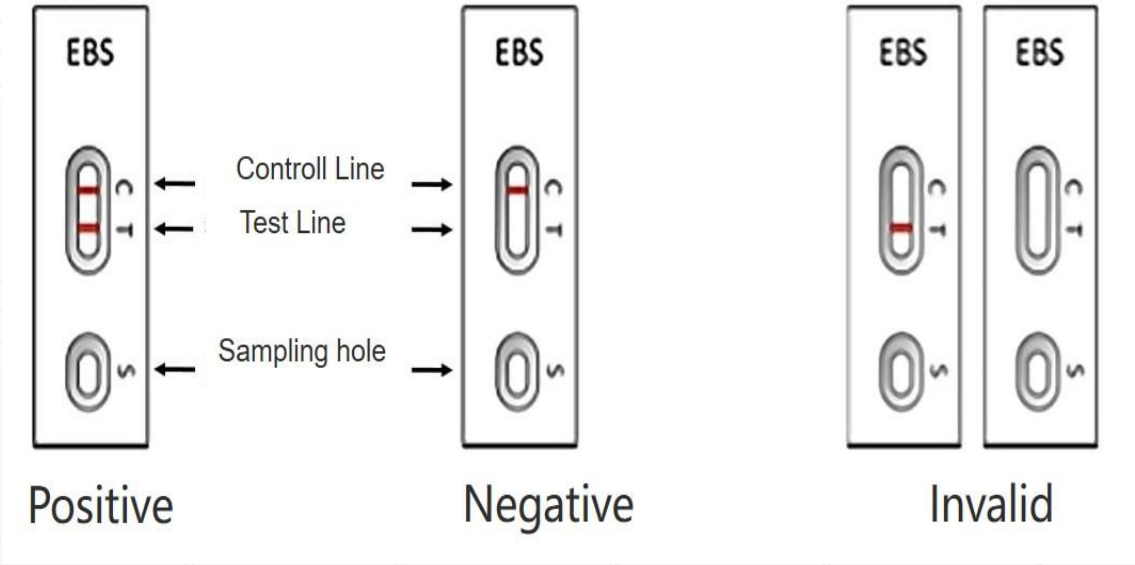
Q : Step 1 2 3, after third step you get the result!

- A : After 10 minutes, the result is available for interpretation. When sample contains the antibody against ASFV P30, the nano-colloidal gold particles will present a red signal on the T line (detection line), which is recognized as a positive result; if the sample does not contain the antibody against ASFV P30, T line (detection line) will not show any color, which is a negative result.



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Q : Operation video

https://youtu.be/SxpoJEpF7mg

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Q : Is it fine using our own syringe to sample ?

- A：Not recommended. The quantitative pipette in each Sentinel® bag is calibrated to ensure that every sample is at the precise amount. By experience, the usage of any other syringes will lead to excessive blood sample, causing the interference and affecting the accuracy of result.

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Q : Internal quality guarantee ?

- A：Each batch of our production will be tested for intra-batch accuracy (figure 1) and inter-batch test accuracy (figure 2), and a small amount of field tests have also been conducted during the development, our quality is guaranteed.

(Fig. 1) In-batch test accuracy (Within Lot Precision)

	Positive (8xASF)			Negative		
Lot : PD651230	Pre-	Medium	Post-	Pre-	Medium	Post-
Line color degree	1.65	2.25	1.6	0	0.15	0
CV	19.70%			173.20%		
Lot : PD651426	Pre-	Medium	Post-	Pre-	Medium	Post-
Line color degree	1.8	1.25	1.35	0	0	0
CV	19.98%			0%		
Internal standard	≧ 1			≦1		
Result	Meet the standard					

(Fig. 2) Batch accuracy (Different Lot Precision)

	Positive (8xASF)	Negative
PD651230	1.3	0.1
PD651426	1.433333	0.11547
CV	6.90%	10.20%
Internal standard	≧ 1	0
Result	Meet the standard	

From: 20190515 Technical Documents

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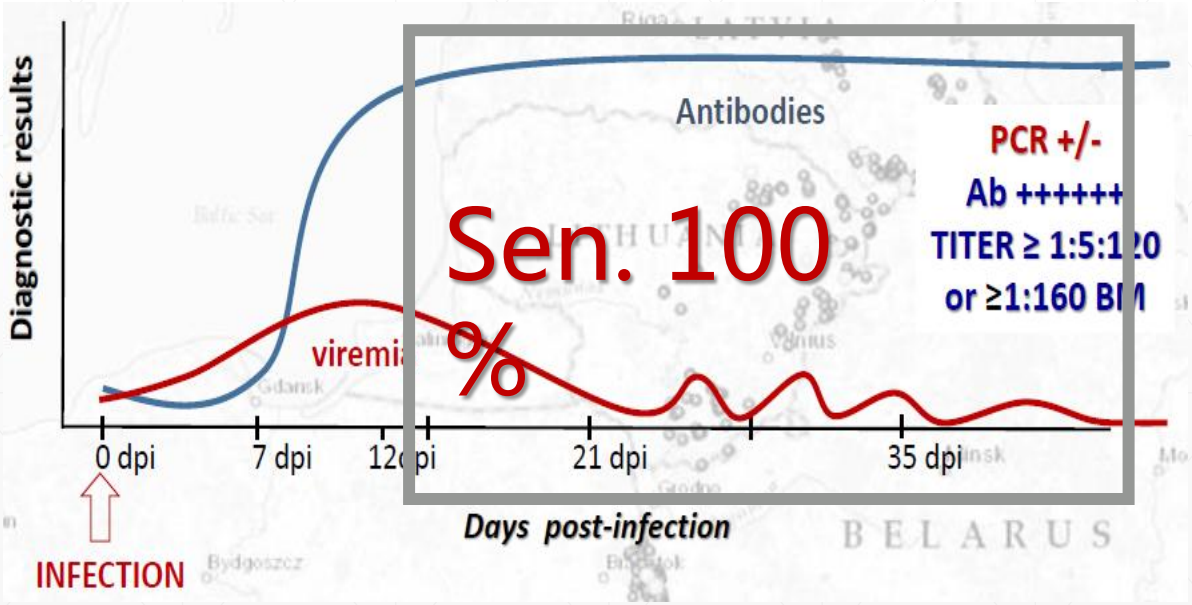
Q : Correct testing time ?

- A : According to the internal efficacy test, it can be tested from 14th to 21st days after infection until the 35th to 42th days. During this infection period the sensitivity reached 100%!



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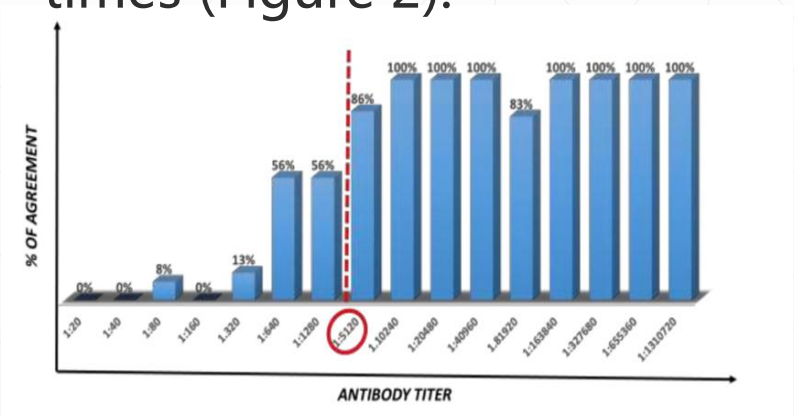
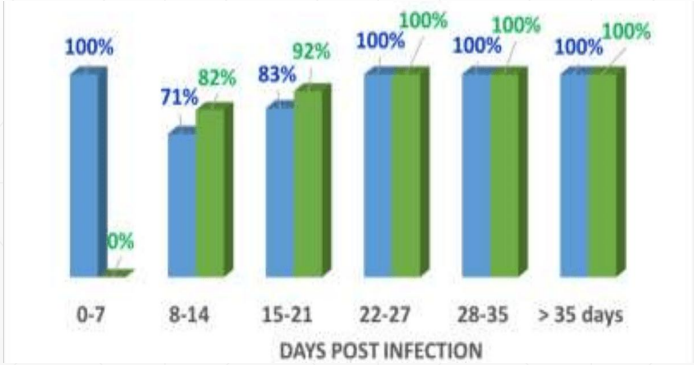
Q : How about Sentinel® detect ASFV in the acute stage ?

- A : The **positive result** of Sentinel® is counted for antibody titer in EU-RL and the **antibody concentration is more than 5120 times** (Figure 1). Pigs in acute stage of ASF infection usually dies in about one week: the antibody concentration in the blood is <5120 times, so it is not easy to detect; but because the incubation period of ASF infection is 4 to 19 days, if the acute stage of the disease shows late, after 8 days, it can be detected, for the antibodies in blood rises rapidly after 7 days, and after 14 days their concentration will be> 5120 times (Figure 2).



(Figure 1) Cut-off point of Sentinel® African Swine Fever Virus Antibody Rapid Test



(Figure 2) Duration of ASF infections, Sentinel® and commercial ELISA are both compared with IPT.