Evaluation of a Portable Point-of-Care Test to Detect African Swine Fever Virus using EDTA Blood and Serum Samples from Pigs at Different ASF Clinical Forms.

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Abstract

Global outbreaks of African swine fever (ASF) have caused a huge economic loss in the pork industry and a health burden on domestic pigs and wild boars in recent years. Here, we demonstrated diagnostic sensitivity of a loop-mediated isothermal amplification (LAMP) based test using 95 experimental blood samples obtained from the EURL-ASF sample bank. This technique has great potential to realize low-cost, high accuracy point-of-care (POC) field testing of ASFV.

Introduction

This study, conducted in CISA-INIA, evaluates the performance of the ZYTCA Ulfa™ ASFV test

- 95 ASFV (+/-) pig whole blood and serum samples
- Analytical sensitivity and specificity benchmarked against the WOAH-validated real-time PCR (UPL) (Fernández et al., 2013).

- ✓ Portable (*14.2 cm*7.2cm, 500g)*
- ✓ *DNA extraction is not required*
- ✓ *Easy operation*
- ✓ *Patented technology to prevent false positive*

Figure 1: Test flow of UlfaQ™ ASFV

Materials and Methods

ASFV Strain	P72 Genotype	Origin	Clinical form	Virulence Designation*	Days post infection (DPI)	# of blood tested
Arm07	II	Armenia 2007	acute	vir	7-36	6
E75	I	Spain 1975	acute	vir	7	2
E75	I	Spain 1975	subacute	mod vir	14-15	2
Est15/WB-Valga6	II	Estonia 2015	subacute	mod vir	25	1
Est16/WB-VIRU8	II	Estonia 2016	acute	mod vir	15	1
Est16/WB-VIRU8	II	Estonia 2016	subacute	mod vir	72	1
ET13/1505	XXIII	Ethiopia 2013	acute	mod vir	10	1
ET13/1505	XXIII	Ethiopia 2013	subacute	mod vir	23-93	2
Ken05/Tk1	X	Kenya 2005	subacute	mod vir	70	2
Ken06/Bus	IX	Kenya 2006	acute	vir	12	1
Lt14/1490	II	Lithuania 2014	acute	vir	17-21	5
Lt22/WBTAU11	II	Lithuania 2022	acute	acute	9-16	4
LV17/WB/RIE1	II	Latvia 2017	chronic	Att	10-20	5
LV17/WB/RIE14/TU KUMA5	II	Latvia 2017	subacute	mod vir	119	1
Lv17/WB/Zieme3	II	Latvia 2017	acute	vir	9-16	4
Lv19/WB/ Brocenu6	II	Latvia 2019	chronic	mod vir	30	1
NHP/68	I	Lisbon 1968	chronic	att	51-126	4
Ourt88/3	I	Portugal 1988	chronic	att	22	1
POL18/WB CASE1865	II	Poland 2018	subacute	vir	18	1

*Table 1: A total of 45 pig experimental blood samples used for the determination of the analytical sensitivity. *Att.= attenuated; vir.= virulent, mod. vir.= moderate virulent*

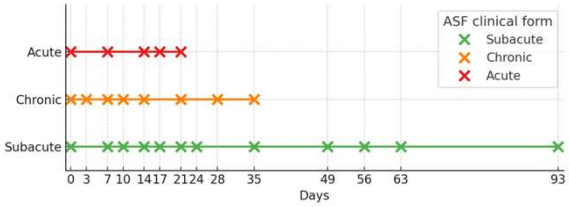

Figure 2: 50 experimental samples (25 serum and 25 EDTA-blood paired samples) throughout the course of infection were analysed in this study.

References

Fernández-Pinero, Jovita, et al. "Molecular diagnosis of African swine fever by a new real-time PCR using universal probe library." *Transboundary and emerging diseases* 60.1 (2013): 48-58.

Carrillo, C., et al. "Long-term persistent infection of swine monocytes/macrophages with African swine fever virus." *Journal of virology* 68.1 (1994): 580-583.

Results

The ZYTCA Ulfa™ ASFV test demonstrated an overall analytical sensitivity of **90.5% (67/74)** when taking out weak-positive samples (C_T > 35). Analytical specificity was high at **95% (20/21).**

C_T Value Range	UPL-PCR	Ulfa™ ASFV	Analytical Sensitivity			
	# of Positives		Total	C_T<40	C_T<35	C_T<30
< 20	17	15	88%	86%	92%	94%
≥ 20 and < 25	4	4	100%			
≥ 25 and < 30	10	10	100%			
≥ 30 and < 35	8	7	88%			
≥ 35 and < 40	5	2	40%			

Table 2: Correlation among the percentage of sensitivity of the Ulfa™ ASFV rapid test and the C_T value ranges obtained with the UPL real time PCR in 45 experimental blood.

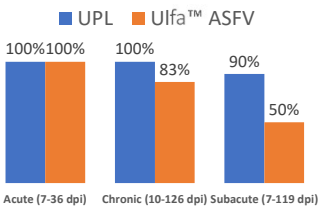

Figure 3: Correlation among the percentage of positive samples with regards to ASFV clinical form (acute: n=23, chronic: n=12, subacute: n=10) obtained with the UPL-real time PCR and Ulfa™ ASFV Kit

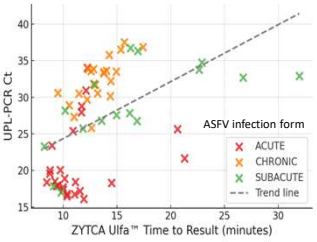

Figure 4: Correlation ($r = 0.506$, $R^2 = 0.256$, $p < 0.001$) of UPL-PCR Ct value and Ulfa™ ASFV time to result using fluorescence signal of 63 ASFV-positive samples.

The comparative study of 50 paired blood and serum samples (Table 3) revealed similar sensitivity in detecting antigens against ASFV in serum (79%) versus blood samples (80%).

ASFV Strain	P72 Genotype	Clinical Form	DPI/DPE	Blood		Serum	
				UPL	Ulfa™	UPL	Ulfa™
Lv19/WB/DOBEL9 (EURL 2023)	II	Acute	0	40	NEG	40	POS
			7	40	NEG	40	NEG
			14	40	NEG	39.14	POS
			17	34.05	POS	30.94	POS
			21	17.34	POS	17.7	POS
POL18/WB CASE1794 (EURL 2021)	II	Chronic	0	40	NEG	40	NEG
			3	40	NEG	40	NEG
			7	30.15	POS	30.59	POS
			10	33.66	POS	28.92	POS
			14	32.21	POS	30.52	POS
			21	36.89	NEG	33.32	POS
			28	36.51	POS	33.61	POS
			35	39.05	NEG	35.79	NEG
ET13/1505	XXIII	Subacute	0	40	NEG	40	NEG
			7	40	NEG	40	NEG
			10	40	NEG	40	NEG
			14	26.79	POS	27.56	POS
			17	26.78	POS	27.82	POS
			21	32.93	POS	33.88	NEG
			24	32.71	NEG	40	NEG
			35	33.77	POS	40	NEG
			49	34.77	NEG	37.7	NEG
			56	34.83	NEG	37.42	NEG
			63	35.94	NEG	40	POS
			93	40	NEG	40	NEG

Table 3: The results of evaluating Ulfa™ ASFV in detecting the ASFV genome over the course of infection using 50 paired whole blood and serum samples.

Discussion and conclusion

- Ulfa™ ASFV can detect ASFV genotype I, II, IX, X and XXIII.
- Ulfa™ ASFV can detect ASFV directly from whole blood and serum samples.
- Reliable in detecting acute-stage ASFV infections.
- Lower sensitivity in chronic and subacute samples—likely reflecting a higher proportion of cell-associated virus (Carrillo et al., 1994)
- A simple pre-lysis step to be added to liberate ASFV DNA from infected cells.
- Testing time can be shortened to 25-30 minutes.