Evaluation of a commercial real-time PCR kit to detect African swine fever virus using a variety of clinical samples and direct amplification using porcine serum samples

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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 high diagnostic sensitivity and specificity of a taqman-probe real-time PCR based test using 240 clinical samples with confirmed ASFV status, and its ability of direct testing using porcine serum. This technique has great potential to realize low-cost, high accuracy point-of-care (POC) testing of ASFV.

Introduction

The UlfaQ™ ASFV Test (ZYTCA Ltd., Oxford, UK) is a commercial real-time PCR assay designed to amplify the ASFV P72 gene using primers and double-quenched probes, featuring:

- Limit of detection (LoD): 2 copies/reaction or 100 copies/mL
- Protects polymerase from PCR inhibitors (Table 1)[1,2]
- Inclusive of Internal control
- Lyophilised reagents

The aim of this study is to test UlfaQ™ ASFV's diagnostic sensitivity and specificity using clinical samples with confirmed ASFV status, and the ability of direct testing using serum without DNA extraction.

PCR Inhibitor	Samples containing the inhibitor	Concentration that would inhibit PCR	UlfaQ™'s tolerable concentration
Haemoglobin	Whole blood	20µg/reaction	50µg/reaction
Heparin sodium	Whole blood, serum, plasma	0.005 IU/reaction (0.5µg/reaction)	0.01 IU/reaction (1µg/reaction)
Humic acid	Environment swabs, anal swabs, faeces	10ng/reaction	500ng/reaction
IgG	Serum, tissue samples	0.2µg/ml	0.8µg/ml
NaCl	Whole blood, serum, plasma, nasal discharge, urine, or any other body fluid samples	0.2%	0.4%
SDS	Post-lysis clinical samples	0.01%	0.1%

Table 1: Overview of UlfaQ™ reagent tolerance to common PCR inhibitors.

Materials and methods

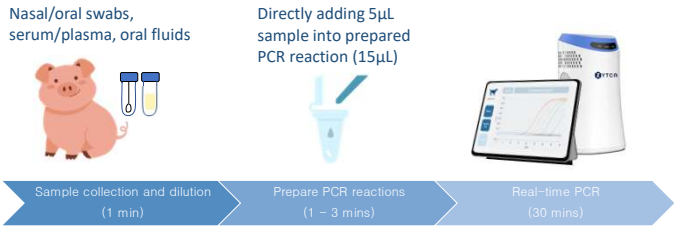
Three independent clinical validation studies were conducted using 240 pig samples. All samples were purified using commercial DNA extraction kit before tested using UlfaQ™ ASFV.

	ASFV + sample	ASFV - sample	Lab location	Comparison test method
Study A	16	13	China	IDEXX RealPCR ASFV
Study B	11	0	China	ID Gene™ ASFV
Study C	94	106	UK (Pirbright Institute)*	WOAH recommended protocol (King et al.)[3]
Total	**123**	**119**		

Table 2: overview of sample numbers, status of ASFV infection, and experiment location of studies conducted.
* For Study C, the samples were originated outside the UK, but the experiments were conducted at the Pirbright Institute.

Additionally, the direct amplification protocol (Figure 1) was evaluated using four ASFV negative porcine serum samples spiked with ASFV P72 plasmids and compared to the standard protocol (DNA was extracted using ReliaPrep™ Blood gDNA Miniprep System, Promega) by Ct values. Three replicates were performed.

Figure 1: Direct amplification protocol of UlfaQ™ ASFV using clinical samples.



Results

The studies (Table 3, 4 and 5) demonstrated that the UlfaQ™ ASFV Test has a diagnostic sensitivity of **97.6% (120/123, 95% CI=93.04% - 99.49%)** and specificity of **94.9% (113/119, 95% CI=89.35% - 98.13%)** across various sample types.

Study A (n=29)

Sample info			UlfaQ™ ASFV Ct value		ID Gene™ ASFV Ct value	
Sample type	ASF status	Other disease status	ASFV (FAM)	IPC (HEX)	ASFV (FAM)	IPC (HEX)
Pig serum	+		26.07	25.91	26.04	28.14
Pig serum	+		21.76	31.04	20.99	30.03
Purified DNA	+		34.59	27.55	34.54	27.37
Purified DNA	+		34.12	29.81	37.18	28.06
Purified DNA	+		34.37	27.03	34.55	27.32
Purified DNA	+		34.24	26.98	34.71	27.54
Purified DNA	+		35.27	26.30	34.54	26.5
Purified DNA	+		21.21	23.17	20.78	23.27
Purified DNA	+		30.69	29.18	29.84	28.99
Purified DNA	+		29.44	31.58	29.6	30.76
Purified DNA	+		39.09	30.59	37.23	29.81
Purified DNA	+		30.47	24.81	29.15	24.59
Purified DNA	+		32.7	27.30	32.38	27
Pig swab in saline	+	PCV +	15.36	20.49	15.96	20.88
Pig swab in saline	+	PRRSV +	34.83	28.47	39.42	27.62
Pig swab in saline	+	PRRSV +	20.42	23.97	20.29	24.48
Pig serum	-		ND	ND	ND	31.85
Pig serum	-		ND	27.94	ND	28.91
Pig serum	-		ND	28.42	ND	30.09
Pig serum	-		ND	26.22	ND	27.48
Pig serum	-		ND	29.09	ND	31.16
Pig serum	-		ND	26	ND	27.6
Pig serum	-		ND	27.5	ND	28.47
Pig swab in saline	-	PCV+	ND	27.38	ND	27.82
Extracted RNA	-	CSF+	ND	23.32	ND	23.6
Extracted RNA	-	PRRSV+	ND	35.85	ND	34.64
Extracted DNA	-	MHP+	ND	ND	ND	35.55
Extracted DNA	-	PRV+	ND	22.37	ND	22.7
Extracted DNA	-	PCV2+	ND	ND	ND	ND

Table 3: Ct values of samples tested in study A using UlfaQ™ ASFV and comparison tests

Study B (n=11)

Sample type	ASF status	Other disease status	UlfaQ™ ASFV Ct value		IDEXX RealPCR Ct value	
			ASFV (FAM)	IPC (HEX)	ASFV (FAM)	IPC (HEX)
Nasal/throat swabs	+		34.44	28.58	33.59	NA
Blood swabs	+		21.41	26.32	20.69	NA
Nasal/throat swabs	+		26.44	27.78	25.43	NA
Blood swabs	+		22.27	26.07	21.47	NA
Blood swabs	+		38.58	26.98	37.5	NA
Blood swabs	+		19.11	26.73	18.4	NA
Environment swabs	+		36.46	28.52	35.45	NA
Environment swabs	+		19.08	22.72	17.93	NA
Blood swabs	+		28.41	25.79	26.28	NA
Nasal/throat swabs	+		21.13	27.32	20.44	NA
Blood sample	+		31.53	28.31	30.61	NA

Table 4: Ct values of samples tested in study B using UlfaQ™ ASFV and comparison tests

Study C (n=200)*

*These data have been generated by The Pirbright Institute and only form part of a larger development and validation program of this assay. The Pirbright Institute has provided the data as is, without any further interpretation or recommendation, and as such does not necessarily endorse the assay.

	Ct range	UlfaQ™ ASFV	King et al.	Agreement
Total Negatives	**No Ct**	**100**	**106**	**94.3%**
Total positives	**All Ct**	**91**	**94**	**96.8%**
Strongly positive	Ct < 20	45	45	100%
Medium positive	Ct ≥20, <28	30	30	100%
Weakly positive	Ct ≥28, <35	13	13	100%
	Ct ≥35	3	6	50%

Table 5: Ct value ranges and test results agreement of UlfaQ™ ASFV with comparison tests.

Standard protocol v.s. Direct amplification of serum samples

Figure 2: Comparisons of C_T values using four porcine serum samples spiked with ASFV P72 plasmid (1k copies/reaction) between different sample pretreatment protocols.

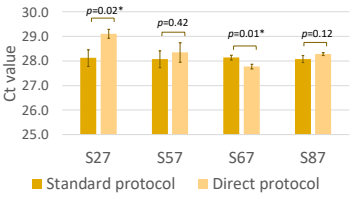


Table 6: C_T values of four porcine serum samples spiked with different concentrations of ASFV P72 plasmid.

Copies/reaction	S27	S57	S67	S87
1000	27.74	27.54	27.77	27.50
	27.52	27.43	27.47	27.32
	27.58	27.35	27.52	27.68
50	35.02	35.99	35.69	36.82
	35.04	36.53	34.76	35.31
	37.34	35.25	34.32	No Ct
2	37.93	No Ct	34.61	37.87
	39.01	No Ct	35.86	37.69
	35.32	37.12	36.98	35.26
0	No Ct	No Ct	No Ct	No Ct
	No Ct	No Ct	No Ct	No Ct
	No Ct	No Ct	No Ct	No Ct

Two porcine samples (S27 and S67) show significant difference of Ct values between the standard and the direct protocol. However, all Ct differences are less than 1, indicating minor impact on sensitivity. Most amplifications with lower copies of targets are successful.

Discussion and conclusion

- UlfaQ™ ASFV Test offers a reliable, sensitive, and efficient diagnostic solution for ASFV detection.
- UlfaQ™ ASFV Test can directly test serum samples, but more clinical samples should be tested to validate the performances.

References

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GARA Global African Swine Fever Research Alliance

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