Development of an Indirect ELISA Using African Swine Fever p11.5 as an Antigen

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Introduction

African swine fever (ASF) has been spreading worldwide. Serological diagnosis methods play a crucial role in ASF prevention and surveillance. We aimed to develop a new indirect enzyme-linked immunosorbent assay (ELISA) based on p11.5, a structural protein of the African swine fever virus (ASFV).

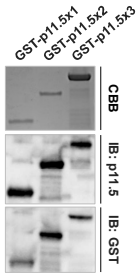
Methods/Results

1. p11.5 Protein as a Target Antigen for Indirect ELISA

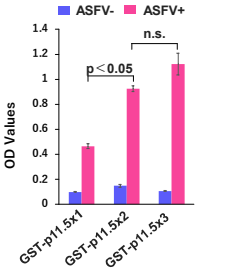
Development of the constructs.

p11.5 protein is encoded by ASFV A137R.
Constructs expressing single and tandemly connected double and triple p11.5 with an N-terminal GST tag were designed as indicated.

The three types of p11.5 target proteins were expressed in *E. coli* and affinity column purified.



Evaluation of antigen properties

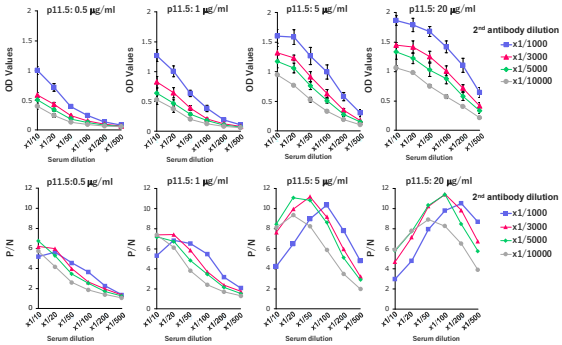


ASFV-: ASFV negative serum
ASFV+: ASFV positive serum

GST-p11.5×3 (hereafter referred to as 'p11.5') was selected as the target antigen for the solid-phase ELISA.

2. Optimization of the Working Conditions of p11.5 ELISA

Determination of an appropriate amount of p11.5 protein for coating and optimal concentrations for test samples and the secondary antibody.



Antigen concentration		Serum dilution 1:10	1:20	1:50	1:100	1:200	1:500
20 μg/ml	P	1.336	1.226	1.028	0.876	0.577	0.331
	N	0.230	0.159	0.100	0.077	0.068	0.058
	P/N	5.81	7.73	10.31	11.38	8.44	5.74
5 μg/ml	P	1.171	1.062	0.761	0.510	0.277	0.152
	N	0.139	0.096	0.070	0.059	0.054	0.053
	P/N	8.42	11.06	10.82	8.60	5.10	2.88
1 μg/ml	P	0.637	0.470	0.285	0.177	0.107	0.071
	N	0.089	0.070	0.059	0.051	0.049	0.046
	P/N	7.19	6.75	4.85	3.48	2.17	1.54
0.5 μg/ml	P	0.506	0.341	0.188	0.128	0.081	0.059
	N	0.075	0.064	0.054	0.050	0.048	0.047
	P/N	6.75	5.30	3.48	2.54	1.71	1.26

α-swine secondary antibody, 1:5000, P: ASFV positive serum, N: ASFV negative serum

Optimal conditions were decided.
Antigen: 50 μl of 20 μg/ml p11.5, Serum dilution: 1:20, Secondary antibody dilution: 1:5000

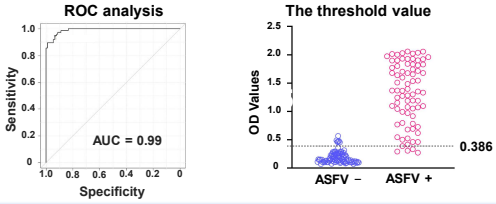
3. Overview of p11.5 ELISA Performance Using Pig Serum Experimentally Infected with ASFV

Pigs were inoculated with ASFV genotypes I, II, and X. Serum was collected every 7 days.

		Group	n	Virus
Genotype	I	1	7	OURT88/3
		2	3	Lisbon60V
	II	3	12	AQS attenuated/ AQS WT
		4	20	Arm07 Δ11MGF
	X	5	6, 2*	Kenya05/TK1

*, Cohabitated wild boar.

4. Standardization and Determination of the Threshold for p11.5 ELISA by ROC Analysis



AUC = 0.99 (95% CI: 0.982–0.999) **Threshold value = 0.386**

5. Comparison between the Conventional ELISA and p11.5 ELISA

The performance of p11.5 ELISA was evaluated using the results of the conventional ELISA as a reference.

		p11.5 ELISA Positive	Negative	Total
Conventional ELISA	Positive	71	5	76
	Negative	5	85	90
	Total	76	90	166

Relative sensitivity = 93.4% (71/76), relative specificity = 94.4% (85/90).

p11.5 ELISA shows high sensitivity and specificity.

6. Reproducibility Evaluation of p11.5 ELISA

Intra- and inter-assay CV% were assessed using five ASFV-positive and five ASFV-negative serum. CV% of ＜10.0 indicates high reproducibility.

Samples	Intra-Assay Mean OD Value	Intra-Assay CV%	Inter-Assay Mean OD Value	Inter-Assay CV%
Negative	0.141	6.39	0.142	2.01
	0.107	1.58	0.103	8.7
	0.109	2.64	0.120	8.79
	0.084	0	0.098	2.09
	0.087	2.48	0.092	4.57
Positive	1.440	3.09	1.440	1.95
	1.390	4.86	1.630	5.54
	0.919	8.81	0.908	2.59
	1.430	5.06	1.460	5.55
	0.665	2.09	0.780	8.75

p11.5 ELISA shows high reproducibility.

7. Evaluation of Antibody Detection Capability of p11.5 ELISA

Comparison of the performance of p11.5 ELISA with the conventional ELISA in detecting specific antibodies in ASFV-infected pig serum

	Genotype I Group 1 n=7	Group 2 n=3	Genotype II Group 3 n=12	Group 4 n=20	Genotype X Group 5 n=6, 2*
Conventional ELISA	7	3	3	15	1
p11.5 ELISA	7	3	4	16	3, 1*

*, A result obtained from a cohabitated wild boar.

p11.5 ELISA can detect antibodies against ASFV genotypes I, II, and X.

8. Comparison of Detectable Time Point.

Comparison of days to first positive detection between conventional ELISA and p11.5 ELISA

	Genotype I Group 1 n=7	Group 2 n=3	Genotype II Group 3 n=12	Group 4 n=20	Genotype X Group 5 n=6, 2*
Conventional ELISA	17	15	39	23.7	21
p11.5 ELISA	15	7	38	19.1	21, 14*

*, A result obtained from a cohabitated wild boar.

The samples from Groups 1-4 (genotypes I, II) showed earlier ASFV antibody detection with p11.5 ELISA.

Conclusion

- ✓ We developed a novel indirect ELISA for ASF serodiagnosis, utilizing the ASFV-derived p11.5 protein.
- ✓ The performance of p11.5 ELISA was comparable to or better than that of a widely used conventional ELISA.
- ✓ This assay enables early and reliable detection of ASFV-specific antibodies in infected pigs and diagnoses the infection caused by various ASFV genotypes.
- ✓ This assay enables early and reliable detection of ASFV-infected animals and has the potential to identify infections caused by various ASFV genotypes.

Reference

M. Watanabe et al., Development of a novel indirect ELISA for the serological diagnosis of African swine fever using p11.5 protein as a target antigen. 2023. *Pathogens*, 12 (6): 774.

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The authors have no COI to disclose.