Optimizing the whole ASFV Indirect ELISA Workflow: A Focus on Pre-Assay Parameters

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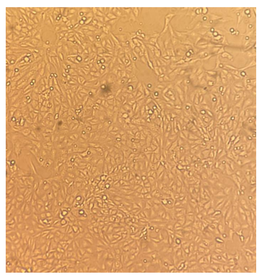
African swine fever virus (ASFV) is a large, enveloped DNA virus that causes a hemorrhagic disease in pigs and wild boars with high lethality. It is endemic in Africa and there have been several outbreaks in Eastern Europe, Asia and the Caribbean making it a real threat to the pig farms around the world. The recently developed vaccine ASFV-G-ΔI177L has shown a solid protection against its parental virus ASFV-G and it is now being tested on its cross protection against different field isolates. This study aimed to enhance antibody detection against African swine fever virus (ASFV) through optimization of the pre-ELISA workflow for an indirect ELISA. The approach focused on (1) identifying robust negative and positive control sera, (2) establishing an ideal antigen coating concentration and (3) determining the optimal antigen harvest time using growth kinetics for the field strains Malawi Lil20/1, Georgia10, Ghana2014, and Ken1033 in wild boar lung (WSL) cells. With an emphasis on replacing primary porcine cells with sustainable WSL cells, the experiment addresses the pressing need for improved diagnostic strategies against this devastating disease.

1 Materials and Methods

Cell Cultures



WSL cells

Vs

PAM cells

Virus Strains

Ken1033, Malawi Lil20/1, Ghana2014, Georgia10

Control Sera Selection

Sera from vaccinated and non-vaccinated pigs tested by indirect ELISA

Antigen Coating Concentrations

Tested 0.5 µg/ml, 1 µg/ml, and 5 µg/ml

qPCR Growth Kinetics

Sampling at 2, 6, 24, 48, 72h
Quantified DNA from cell lysates and supernatant

3 Discussion

WSL cells are a stable alternative to PAMs for ASFV antigen production.

Lower antigen coating (0.5 µg/ml) is effective and reduces reagent use.

Field strains need adaptation to improve WSL growth.

24h is the best time point for harvesting ASFV for ELISA use.

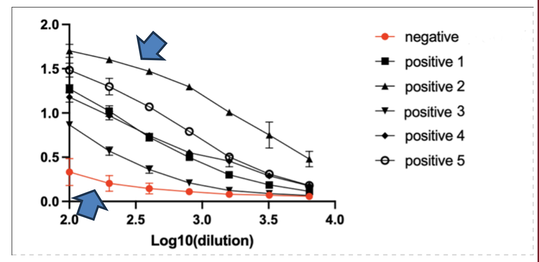
2 Results

ELISA OD Values with clearest signal difference

Negative

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,225	0,44	1,24	1,305	1,651	1,755	0,849	0,882	1,138	1,219	1,541	1,429
B	0,142	0,267	1,063	0,978	1,589	1,616	0,536	0,602	0,936	1,002	1,364	1,232
C	0,103	0,188	0,757	0,695	1,479	1,464	0,331	0,392	0,727	0,761	1,059	1,079
D	0,086	0,136	0,504	0,49	1,296	1,295	0,193	0,225	0,529	0,571	0,795	0,785
E	0,068	0,095	0,291	0,312	0,981	1,033	0,119	0,128	0,411	0,502	0,486	0,518
F	0,069	0,074	0,181	0,193	0,647	0,854	0,086	0,093	0,283	0,297	0,297	0,322
G	0,054	0,06	0,112	0,117	0,418	0,54	0,065	0,071	0,169	0,182	0,169	0,194
H	0,045	0,043	0,047	0,048	0,044	0,043	0,043	0,043	0,047	0,048	0,044	0,046

Positive 2 = PU078



Protein concentration (µg/ml) from WSL vs. PAM

Samples	OD	Concentration mg/ml
Malawi WSL	0.207	0.4584
Georgia WSL	0.137	0.2876
Georgia PAMs	0.1275	0.2644
Malawi PAMs	0.1305	0.2717

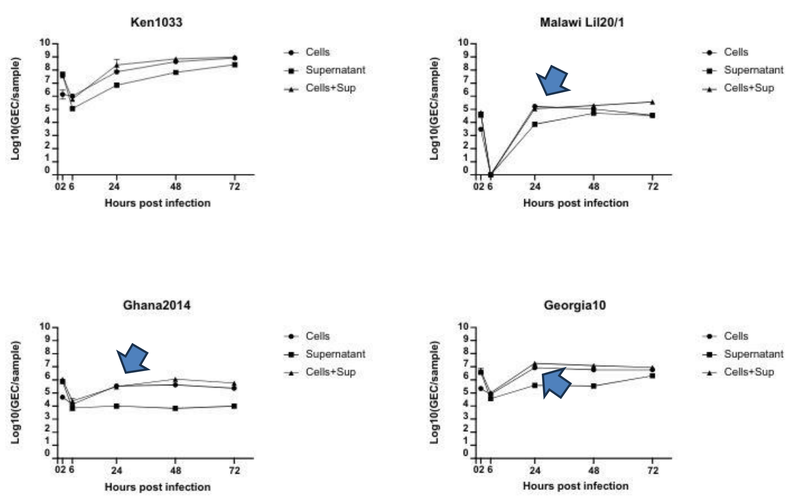
Samples	OD	Concentration mg/ml
ASFV Malawi	0.24	0.5013345383
ASFV Georgia	0.207	0.4232787374
ASFV Ghana	0.201	0.4090867736

Optimal antigen coating (strong signal, minimal noise)

	0.5 ug/ml				1 ug/ml				5 ug/ml			
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.351	0.326	0.816	0.756	0.483	0.386	0.589	0.594	0.242	0.212	0.33	0.338
B	0.225	0.264	0.568	0.554	0.39	0.276	0.433	0.49	0.198	0.159	0.225	0.225
C	0.199	0.19	0.471	0.415	0.216	0.196	0.361	0.337	0.196	0.177	0.167	0.175
D	0.231	0.152	0.302	0.27	0.163	0.152	0.243	0.237	0.107	0.132	0.14	0.137
E	0.151	0.117	0.22	0.188	0.103	0.094	0.185	0.222	0.098	0.076	0.098	0.098
F	0.083	0.079	0.148	0.165	0.096	0.079	0.151	0.139	0.112	0.071	0.086	0.077
G	0.061	0.059	0.123	0.13	0.082	0.106	0.125	0.115	0.07	0.058	0.069	0.064
H	0.154	0.046	0.055	0.051	0.049	0.057	0.054	0.067	0.052	0.084	0.046	0.047

Peak viral growth at 24h post-infection

Strain	Growth	2h	6h	24h	48h	72h	Ratio Kenya 24h
Malawi Lil20/1	Cells	3.07E+03	1.07E+03	1.71E+05	1.05E+05	3.54E+04	430.66
Malawi Lil20/1	Cells+sup	5.77E+04	2.34E+03	1.14E+05	5.69E+06	3.75E+05	
Malawi Lil20/1	Sup	4.3E+04	1.01E+03	7.38E+03	3.96E+05	3.48E+04	
Georgia10	Cells	2.19E+05	8.04E+04	8.49E+06	5.92E+06	5.76E+06	8.73
Georgia10	Cells+sup	5.4E+06	1.01E+05	1.83E+07	1.22E+07	8.78E+06	
Georgia10	Sup	3.85E+06	3.74E+04	3.87E+05	3.43E+05	2.07E+06	
Ghana2014	Cells	4.83E+04	1.8E+04	3.52E+05	4.19E+05	2.31E+05	238.23
Ghana2014	Cells+sup	1.13E+06	2.52E+04	3.05E+05	1.13E+06	5.63E+05	
Ghana2014	Sup	7.43E+05	7.20E+03	1.06E+04	6.90E+03	9.83E+03	
Ken1033	Cells	1.59E+06	1.03E+06	7.38E+07	4.4E+08	8.33E+08	1
Ken1033	Cells+sup	3.75E+07	6.09E+05	3.03E+08	7.24E+08	9.80E+08	
Ken1033	Sup	4.9E+07	1.15E+05	7.06E+06	6.89E+07	2.57E+08	



4 Conclusions

- A new indirect ELISA protocol for ASFV antibody detection was successfully developed.
- Reliable positive and negative control sera were identified to improve result interpretation.
- The optimal viral harvest time was established at 24 hours post-infection.
- WSL cells proved effective for ASFV antigen production, offering a practical alternative to PAMs.
- Further fine-tuning of antigen concentration and viral adaptation is needed to improve sensitivity.
- This optimized ELISA contributes to better disease monitoring and vaccine evaluation strategies.

References

Blome S, Mettenleiter T C, Williams D T. 2024. African swine fever: advances and challenges. Revue Scientifique et Technique de l'OIE. Special Edition:58–69. doi:https://doi.org/10.20506/rst.se.3559.

Borca MV, Rai A, Espinoza N, Ramirez-Medina E, Spinard E, Lauro Velazquez-Salinas, Valladares A, Silva E, Burton L, Meyers A, et al. 2023. African Swine Fever Vaccine Candidate ASFV-G-ΔI177L Produced in the Swine Macrophage-Derived Cell Line IPKM Remains Genetically Stable and Protective against Homologous Virulent Challenge. Viruses. 15(10):2064–2064. doi:https://doi.org/10.3390/v15102064.

Dinhobl M, Spinard E, Birtley H, Tesler N, Masembe C, Ribeca P, Borca MV, Gladue DP. 2024a. African swine fever virus biotype identification tool. Microbiology Resource Announcements. 14(2):e00530-24. doi:10.1128/mra.00530-24.

Escribano JM, Galindo I, Alonso C. 2012. Antibody-mediated neutralization of African swine fever virus: Myths and facts. Virus Research. 173(1):101–109. doi:https://doi.org/10.1016/j.virusres.2012.10.012.

World Organisation for Animal Health (OIE). African Swine Fever (Infection with African Swine Fever Virus) [Internet]. Paris: OIE; c2021 [cited 2025 Feb 10]. Available from: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.08.01_ASF.pdf

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