

Development of a highly sensitive point-of-care test for African swine fever that combines EZ-Fast DNA extraction with LAMP detection: evaluation using naturally infected swine whole blood samples from Vietnam



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1 Introduction

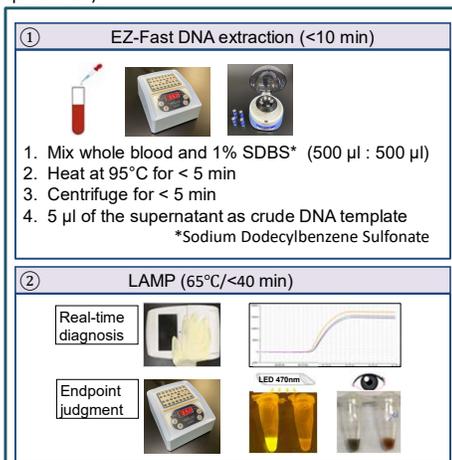
While early detection and early containment are key to controlling the African swine fever (ASF) pandemic, the lack of practical testing methods for use in the field are a major barrier to achieving this feat. Here, we describe the development of a rapid and sensitive point-of-care test (POCT), and its evaluation using clinical samples. We validated a sensitive POCT for ASF in swine whole blood samples for field settings.

Key words

- Simple DNA extraction (EZ-Fast)
- Field setting
- Loop-mediated isothermal amplification (LAMP)
- Point-of-care test (POCT)

2 Methods

In total, 89 swine whole blood samples were collected from 38 ASF outbreak swine farms located in nine provinces in northern Vietnam from June 2020 to January 2022 and were performed the POCT using a combination of EZ-Fast (simple DNA extraction) and LAMP (loop-mediated isothermal amplification).



Design of LAMP primers for ASFV

- Target the genome of p72 protein encoding ASFV capsid.
- Collected 216 p72 protein sequences from GenBank and designed LAMP primers using Primer Explorer Ver.5.
- Confirmed the specificity of the designed primers by BLAST search.
- Confirmed that the primers were negative for three isolates of the causative virus of classic swine fever (CSF), which have similar clinical symptoms to those of ASF.

Comparison of diagnostic performance between the POCT and the conventional real-time PCR

The performance of POCT (EZ-Fast DNA extraction combined with LAMP) was evaluated in comparison to the conventional method which combined real-time PCR with column kit DNA extraction. The limit of detection (LOD) of the conventional real-time PCR method and the POCT for ASF detection were compared using swine whole blood samples.

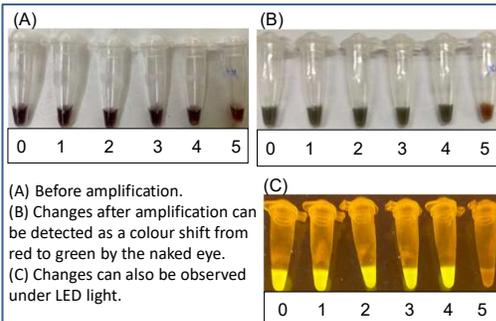
3 Results & Discussion

Table 1. Diagnostic performance of the POCT for ASF.

POCT (EZ-Fast DNA extraction combined with LAMP)	Conventional method (real-time PCR with column kit-extracted DNA)	
	Positive	Negative
Positive	56	0
Negative	0	33

Diagnostic sensitivity: 100% (56/56), Diagnostic specificity: 100% (33/33)

The POCT enabled crude DNA to be extracted from swine whole blood samples within 10 min at extremely low cost and with relative ease. The entire POCT required a maximum of 50 min from the beginning of DNA extraction to final judgment. Compared to a conventional real-time PCR detection, the POCT showed a 1 log reduction in detection sensitivity, but comparable diagnostic sensitivity of 100% (56/56) and diagnostic specificity of 100% (33/33). The POCT was quicker and easier to perform and did not require special equipment.



0, Original ASFV-positive blood sample; 1–5, 10-fold serial dilutions of the ASFV-positive sample in pooled ASFV-negative blood samples.

Figure 2. Limit of detection of the POCT for ASF.

Table 2. Comparison of the limit of detection (LOD) between the conventional real-time PCR and the POCT for ASF.

Dilutions	0	1	2	3	4	5	6
Conventional (Ct)	19.28	20.76	24.30	27.86	31.11	34.99	No. Ct
POCT (Tp)	21:46	22:00	25:44	26:14	29:16	No. Tp	No. Tp

0, Original ASFV-positive blood sample; 1–6, 10-fold serial dilutions of the ASFV-positive sample in pooled ASFV-negative blood samples. Ct, threshold cycle; Tp, time to positivity

4 Conclusion

This POCT is expected to facilitate early diagnosis and containment of ASF invasion into both regions in which it is endemic and eradicated.

5 References & Acknowledgements

Ngan T. M., Yamazaki Y., Yamazaki W. *et al.*, (2023). Development of a highly sensitive point-of-care test for African swine fever that combines EZ-Fast DNA extraction with LAMP detection: evaluation using naturally infected swine whole blood samples from Vietnam. *Veterinary Medicine and Science*, 9(3), 1226-1233. DOI:10.1002/vms3.1124

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