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Development of a highly sensitive and cost-effective point-of-care test for African swine fever with loop-mediated isothermal amplification detection: evaluation using naturally infected swine whole blood samples from Vietnam

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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. Highly sensitive and rapid real-time polymerase chain reaction (PCR) assays are widely used for routine diagnosis of ASF. However, in many countries, animal quarantine stations and regional livestock health centers, which are responsible for waterfront quarantine, do not have sufficient diagnostic facilities and the significant associated cost of equipment and reagents. To describe the development of a sensitive and cost-effective point-of-care test (POCT) for ASF with loop-mediated isothermal amplification (LAMP) detection, and its evaluation using swine whole blood samples for field settings.

Methods
The POCT protocol was followed according to our previously reported methods [1]. In total, 89 swine whole blood samples were collected from Vietnamese swine farms and were performed the developed POCT using a combination of crude DNA extraction by sodium dodecyl benzenesulfonate (SDBS) and loop-mediated isothermal amplification (LAMP). LAMP primers were newly designed based on the p72 protein-encoding region of the ASFV genome. We evaluated diagnostic performance of the POCT in comparison to the conventional method that combines a DNA extraction kit and real-time PCR.

Results
The POCT enabled crude DNA to be extracted from swine whole blood samples within 10 min at extremely low cost less than $0.1 (USD) per sample and with relative ease. The entire POCT required a maximum of 50 min from the beginning of DNA extraction to final judgment by the naked eye through changes in color. 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 easier to perform and did not require special cumbersome equipment.

Conclusions
This POCT is a simple, cost-effective, and practical method with potential application in on-site diagnosis and is expected to facilitate early diagnosis and containment of ASF invasion into both regions in which it is endemic and eradicated.