FAO Innovations: POC assay for outbreak management

Comparative analysis of laboratory-based and portable qPCR platforms for ASF diagnosis on farm

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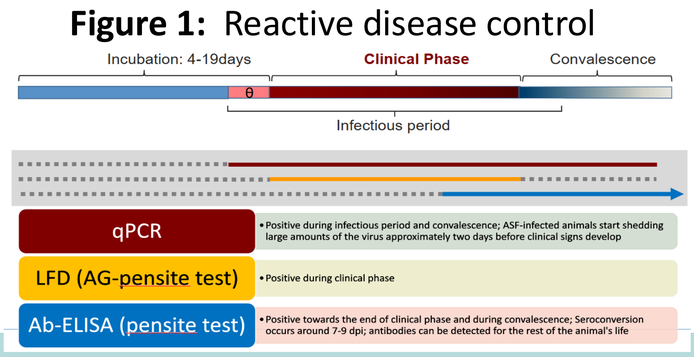
1 Introduction

Rapid and reliable early detection of African Swine Fever Virus (ASFV) is critical for effective outbreak management. Early detection enables swift implementation of biosecurity measures to contain and prevent further spread within and outside the affected premises. This study compares the performance of different qPCR platforms in terms of sensitivity, reproducibility, and field adaptability. DNA detection is vital for identifying the virus during the incubation period, before clinical signs appear, and while animals are shedding large amounts of the virus, allowing for timely intervention and control.

Figure 1: Reactive disease control



- Early ASFV detection is vital for effective outbreak management.
- The study compares qPCR platforms for ASFV detection.
- DNA detection enables timely intervention before clinical signs appear

2 Methods/Approach

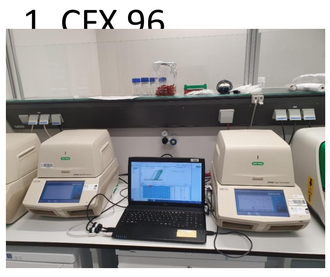
This study evaluated three qPCR platforms for detecting ASFV using pre-extracted viral DNA from 72 ASFV-positive samples from 14 countries (West Africa (2021, 2019, 2017), Central Africa (2017),East Africa (2019, 2016, 2023), Southern Africa (2019, 2023), Southeast Asia (2021, 2023), East Asia (2021), represented ASFV genotypes I, II, IX, XIV, XXIII, and recombinant strains. The platforms were assessed for sensitivity, reproducibility, and field adaptability. The Bio-Rad CFX96 and MIC platforms followed the King et al. 2003 protocol with iTaq master mix, while the Franklin platform was tested with both proprietary lyophilized reagents and iTaq master mix. Negative controls included DNA from other pathogens. All samples were extracted using the DNeasy Blood & Tissue kit. Specificity was tested by including a panel of swine diseases, such as Salmonella spp., PRRSV, SVDV, and others, to ensure no cross-reactivity.

Specifics for each platform:

- CFX96: High-throughput, 96-well system.
- MIC: Compact, 48-sample format with magnetic induction technology.
- Franklin: Portable, battery-operated system with multiplexing capability, 9-well system.

Image 1: PCR-Platforms compared

1. CFX 96



2. MIC PCR

3. Franklin Biomeme

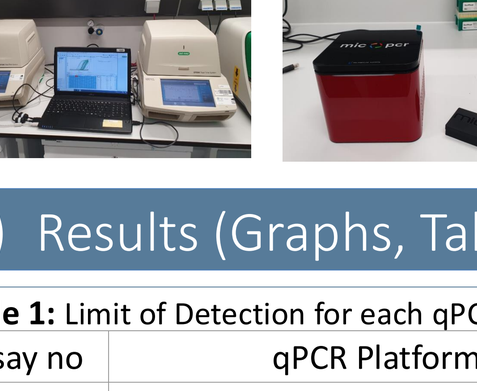
3 Results (Graphs, Tables, Figures)

Table 1: Limit of Detection for each qPCR platform (gene copies/reaction)

Assay no	qPCR Platform	LOD (95% CI)
1	CFX96	21.28 (14.85–68.67)
2	MIC	32.76 (21.12–96.11)
3	Franklin (lyophilized reagents)	4.12 (2.71–14.97)
4	Franklin (adapted iTaq protocol)	6.85 (4.33–20.11)

Figure 2: Bland-Altman plots show the agreement between qPCR platforms and the reference CFX96. The X-axis represents the mean Ct value between the two platforms, while the Y-axis shows the Ct difference (bias). The solid line indicates mean bias, and dashed lines represent the limits of agreement (±1.96 SD). These plots highlight systematic differences and variability between platforms.

2a) Assay 1 compared to assay 2



2b) Assay 1 compared to assay 3

2c) Assay 1 compared to assay 4

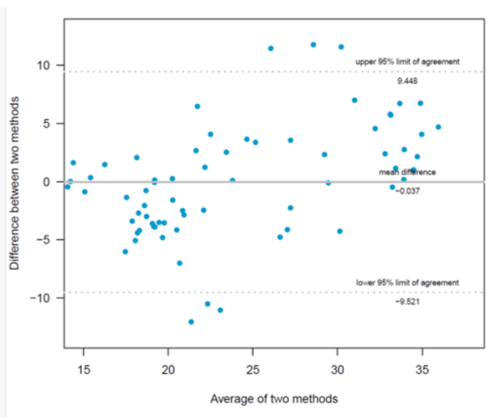


Table 2: Inter-assay agreement calculations using Biorad CFX-platform as the comparator.

Comparison	Spearman's rho (ρ)	Bias (Mean Difference in Ct)	Fleiss' Kappa
Assay 1 compared to assay 2	0.205	2.56	1
Assay 1 compared to assay 3	0.054	-1.594	1
Assay 1 compared to assay 4	0.763	-0.037	1

Field Applications:

Vietnam, April 2024 – During ASF surveillance on a Vietnamese sow farm, assay 2 and 3 enabled early detection of infected animals, guiding selective culling and reducing further virus spread (Nga et al., 2024).

Image 2: Sow-farm

Iringa, Tanzania, June 2023 – During participatory training course on field sampling, storage and laboratory diagnostic techniques for ASF, assays 2 and 3 were successfully applied on an abattoir, providing immediate results in the field.

Image 3: Abattoir

4 Discussion

Portable qPCR systems, like the Biomeme Franklin, enable sensitive, reproducible, rapid, field-based ASFV detection, making them valuable for decentralized diagnostics. However, they exhibit a lower throughput compared to standard laboratory platforms. Despite these limitations, their low power consumption and suitability for resource-limited settings—where portable machines are approximately 25% less expensive than laboratory-scale qPCR systems—make them an ideal choice for outbreak response and early disease detection.

- **Decentralized Diagnostics:** Useful in settings with limited lab infrastructure, personnel and electricity shortages.
- **Outbreak Response & Early Detection:** Effective for on-site disease surveillance and rapid decision-making.

5 Conclusion

Portable qPCR platforms offer sensitive and reproducible results for ASFV detection on-site i.e. farms or abattoir. While they provide rapid results, confirmatory testing in laboratories remains essential. Standardized protocols, improved reagent stability, and workflow optimization are needed to enhance their reliability for surveillance and outbreak response.

6 References & Aknowledgements

References:

1. King, D.P.; Reid, S.M.; Hutchings, G.H.; Grierson, S.S.; Wilkinson, P.J.; Dixon, L.K.; Bastos, A.D.; Drew, T.W. Development of a TaqMan PCR Assay with Internal Amplification Control for the Detection of African Swine Fever Virus. J. Virol. Methods 2003, 107, 53–61.
2. Nga, Bui & Auer, Agathe & Padungtod, Pawin & Dietze, Klaas & Globig, Anja & Rozstalnyy, Andriy & Hai, Tran & Depner, Klaus. (2024). Evaluation of Selective Culling as a Containment Strategy for African Swine Fever at a Vietnamese Sow Farm. Pathogens. 13. 567. 10.3390/pathogens13070567.

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