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Development of a novel African Swine Fever Virus PCR to address current market requirements.

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Background and objectives

African Swine Fever poses an ever-increasing threat to swine populations globally. Fast, accurate, robust diagnostic methods with pooling possibilities are required for detection, mass-screening, surveillance, and monitoring. RT-PCR, the “gold standard” method for detection of ASFV, has limitations including false negative results (inhibition), temperature sensitive reagents and time-consuming protocols. The vetproof® African Swine Fever Virus qPCR LyoKit, a triplex qPCR with precoated lyophilized plates, a double control system (endogenous and exogenous Internal Control) and UNG enzyme to prevent false positives by PCR amplicon contamination. This study evaluates the performance and robustness of this new lyophilized kit against a reference assay.

Materials and methods

Evaluation of the assay requires internal and external validation. Analytical sensitivity, robustness and reproducibility was assessed by Hygiena Diagnostics GmbH on spiked-, negative- and reference material sets provided by CISA-INIA(Spain) and Państwowy Instytut Weterynaryjny (Poland) according to the protocol as per the instructions for use. Applicability on a range of thermal cyclers demonstrated. An external validation performed by The Pirbright Institute (OIE ASF Reference Laboratory). Diagnostic sensitivity and specificity were assessed by 322 defined samples compared to the OIE reference method by King et al. The sample set included ETDA Blood, porcine serum, cell cultured virus, swab samples and homogenized tissue.

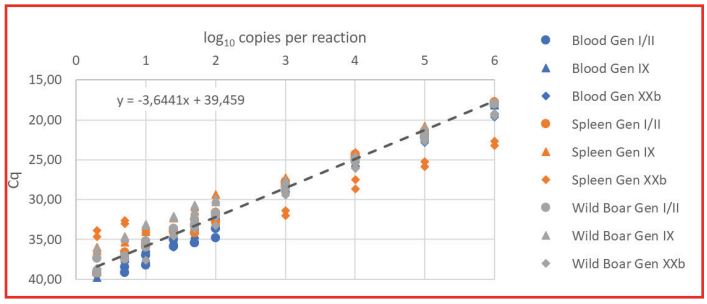
Results

Internal validation shows highly reproducible detection (<2%CV) and a clear dose/response relationship between 1 million to 10 viral genome copies/reaction (Table 1). The LOD95% is approximately 5 copies/reaction. Inclusivity was shown for a diverse range of ASFV genotypes (Table 2) and exclusivity against CSFV and swine-negative matrices. The assay detects ASFV in pooled serum samples (n=10) with high sensitivity. The kit can be paired with PCR standards for precise viral load quantification.
The external validation demonstrates good agreement (97,2%) between the newly developed assay and the King et al. PCR with no obvious difference in Dse (>97%) and Dsp (100%). ASFV was detected in samples ranging from highly to weakly positive for ASFV.

Discussion and Conclusion

The vetproof® African Swine Fever Virus qPCR LyoKit demonstrated perfect suitability for the detection of ASFV genetic material for all relevant sample types. The lyophilized format offers improved storage and handling for the diagnostic laboratory. Possibilities of extending this lyophilized assay to Point-of-Care applications should be further investigated.

Table 1 **Dose response plot**



In all instances the detection was highly reproducible and displayed a clear dose-response relationship between 10 to 1 million viral genome copies per reaction. The LOD95% a measure of the minimal copy number per reaction that will provide a certain positive detection was very low for all matrices and genotypes at approximately 5 copies per reaction.

Table 2 **Inclusivity of ASFV Strains (partial data)**

Strain	Genotype	Result
Arm07L9 (West Africa)	II	Detected
Ken06.Bus (East Africa)	IX	Detected
RSA/3/96 (South Africa)	XIX	Detected
Zaire (Central Africa)	XXb	Detected
NH/P68 (West Europe)	I	Detected
L60 (West Europe)	I	Detected
Ukr12/Zapo (East Europe)	II	Detected
LT14/1490 (East Europe)	II	Detected
Moz64L2 (East Arfica)	V	Detected
Ken05/K2 (East Africa)	X	Detected

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