More control and confidence in the detection of African Swine Fever Virus in difficult sample material

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Introduction

Since 2015, INDICAL's FLI-registered virotype® ASFV PCR Kit Kit (FLI-B 670) has helped to combat African Swine Fever (ASF) in Europe. To better address challenging sample types, the new virotype ASFV 2.0 PCR Kit comes with a double control strategy. The triplex qPCR assay includes an exogenous internal control in the lysis buffer to verify the success of the extraction procedure and an endogenous internal control to verify sample presence and quality. This system offers a higher level of confidence in the interpretation of qPCR results, which is crucial when dealing with difficult sample types.

Workflow

DNA isolation
2 µl of Internal Control DNA per sample is added to the lysis buffer during extraction, which can be performed using spin columns (e.g., IndiSpin® Pathogen Kit) or magnetic beads (e.g., IndiMag® Pathogen Kit). Note that INDICAL provides pre-treatment protocols for tissue and swab samples.

Sensitive qPCR
The virotype ASFV 2.0 PCR Kit is a highly sensitive and easy-to-use multiplex assay for amplifying and detecting ASFV DNA. Its key features are:

- A ready-to-use master mix for convenient assay setup
- An endogenous extraction control (EC) to verify sample presence and quality
- An exogenous internal amplification control (IC) to monitor the success of the extraction procedure and partial inhibition
- Chemistry compatible with all qPCR cyclers commonly used in veterinary labs
- A total amplification time of about 1 hour*

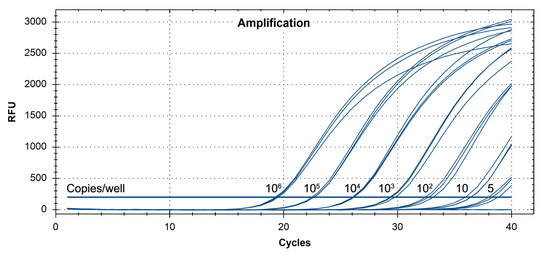
* Based on a performance on the Bio-Rad® CFX96™

High analytical sensitivity

The high analytical sensitivity of the virotype ASFV 2.0 PCR Kit was shown using a titration series of in vitro DNA (10^6–10^0 copies/well) performed in triplicate and analyzed using a Bio-Rad CFX96.

Equal precision and sensitivity were demonstrated using the Stratagene® Mx3005P, Aria MX®, Rotor-Gene® Q and Applied Biosystems® 7500 Real-Time PCR System.

The virotype ASFV 2.0 PCR Kit detected 5 ASFV DNA copies per sample with a correlation coefficient ≥ 0.996 on all instruments tested.



Double control strategy

The double control strategy facilitates the monitoring of extraction procedures, especially in cases of difficult sample material, and helps avoid false negatives. Partial inhibition can be detected when the IC-DNA is added to the lysis buffer during extraction. Difficult ASFV-negative blood samples from wild boar were tested to show the benefit of the double control strategy.

Sample	Material	ASFV C_T (FAM)	EC C_T (HEX)	IC C_T (Cy5)	
Wild boar 1	Blood	–	**27.03**	**29.10**	Poor sample quality
Wild boar 1	Blood	–	35.77	**28.29**	
Wild boar 1	Blood	–	No C_T	**28.14**	
Wild boar 1	blood	–	No C_T	**28.35**	

Sample	Material	ASFV C_T (FAM)	EC C_T (HEX)	IC C_T (Cy5)	
Wild boar 6	Blood	–	34.04	31.09	Partial inhibition
Wild boar 2	Blood	–	36.10	**27.68**	

Sample	Dilution	ASFV C_T (FAM)	EC C_T (HEX)	IC C_T (Cy5)	
Wild boar 6	1:5	–	**32.93**	**29.84**	Partial inhibition

To test the kit's performance against inhibitors, a sample was treated with an increasing concentration of heparin (0.85–17 U/µl).

This inhibition study showed that when inhibition occurs, the internal control signal is the first to decrease.

	Material		
	Heparin (U/µl)	Target (C_T values)	
Samples		**FAM** (ASFV)	**EC** (HEX)	**IC** (Cy5)
1	–	26.08	26.19	29.67
2_1	0.85	26.13	27.18	30.65
2_2	0.85	26.07	27.15	30.48
3_1	1.7	26.18	26.48	32.19
3_2	1.7	26.12	27.03	33.23
4_1	8.5	33.80	–	–
4_2	8.5	35.50	–	–
5_1	17	–	–	–
5_2	17	–	–	–

Strain detection

The virotype ASFV 2.0 PCR Kit detected all samples of the seven genotypes kindly provided by the European Reference Laboratory for ASFV (INIA-CISA): NH/P68 (genotype I), Uk12/Zapo (genotype II), Moz64 (genotype V), MwLil 20/1 (genotype VIII), Ken06.Bus (genotype IX), Ken05/Tk1 (genotype X) and Eth13/1505 (genotype XXIII). The testing of additional genotypes confirmed that all 23 genotypes described can be reliably detected using the virotype ASFV 2.0 PCR Kit.

Cross-reactivity was tested with samples positive for CSFV, PRRSV, SIV and PCV-2. The samples were kindly provided by the FLI and various veterinary diagnostic laboratories. No cross-reactivity for these other porcine viral pathogens was detected.

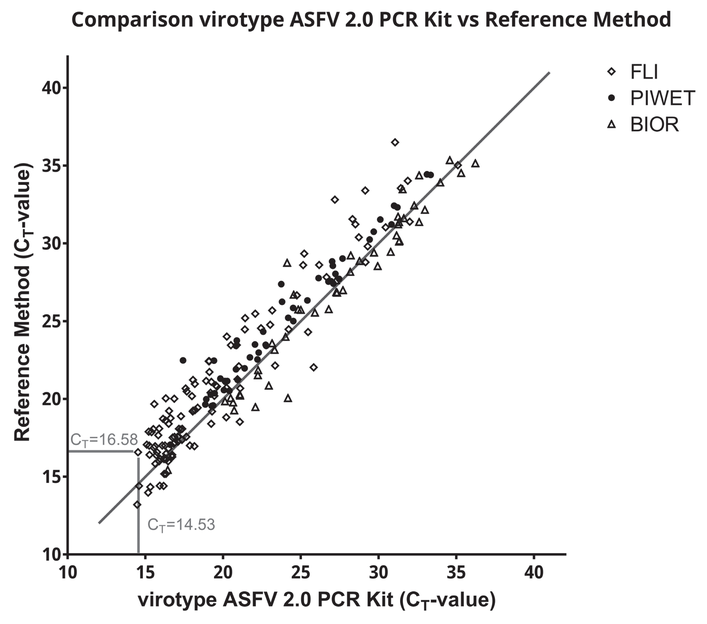
Validation study

Validation of the virotype ASFV 2.0 PCR Kit was performed with 468 reference samples (blood, serum, tissue, fecal and oropharyngeal swabs). 245 ASFV-positive and 11 ASFV-negative samples were kindly provided by INIA, FLI, PIWET and BIOR. The ASFV-positive samples comprised seven different genotypes (I, II, V, VIII, IX, X and XIII). Another 212 ASFV-negative samples were provided by the FLI and other veterinary diagnostic laboratories in Germany.

Viral DNA was extracted using the QIAamp® Viral DNA Mini Kit, the IndiSpin Pathogen Kit, the IndiMag Pathogen Kit or the High Pure Viral Nucleic Acid Kit following the manufacturers' instructions. PCR was performed with the virotype ASFV 2.0 PCR Kit.

virotype ASFV 2.0		Comparative data			
Total	**468**	**Reference positive**	**245**	**Reference negative**	**223**
Positive	245	True positive	245	False positive	0
Negative	223	True negative	0	False negative	223

The virotype ASFV 2.0 PCR Kit demonstrated a diagnostic sensitivity of 100%, a diagnostic specificity of 100% and a diagnostic efficiency of 100%.



Results (C_T values) obtained with commonly used in-house qPCR methods (1, 2) were compared to the results (C_T values) obtained with the virotype ASFV 2.0 PCR Kit.

The virotype ASFV 2.0 PCR Kit showed an equal or better sensitivity compared to the lab-established qPCR methods.

Conclusion

INDICAL's virotype ASFV 2.0 PCR Kit is a reliable and sensitive qPCR assay for the detection of ASFV DNA from serum, plasma, EDTA blood, tissue and swab samples from pigs and wild boars. Its performance is enhanced by its double control strategy. The inclusion of two internal controls reduces the risk of false negative results and helps to verify the success of both extraction and amplification.

FLI-approval pending

References
1. King, D.P., et al. Development of a TaqMan PCR assay with internal amplification control for the detection of African Swine Fever Virus. J Virol Methods. 2003;107(1):53–61.
2. Fernández-Pinero, J., et al. Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library. Transbound Emerg Dis. 2013;60(1):48–58.

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