Evaluation of VDx® ASFV qPCR in Poland

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1. ABSTRACT

African Swine Fever (ASF) is a highly contagious viral disease of suids with severe hemorrhagic fever and high mortality. This disease is endemic in many countries of Africa, eastern Europe and recently in China. Because there is no commercial vaccine or chemotherapeutics available, preliminary quarantine of the disease by fast diagnosis and biosecurity is the only way to control the disease. The real time PCR kit targeting the P72 gene of ASF virus (ASFV) has been commercialized (VDx® ASFV qPCR kit) and the performance of this kit was evaluated. A panel of 6 DNA samples of different genotype derived from EURL in Valdeomos in Spain (ASFV gen. I, II, V, VIII, IX, and X) and a panel of 54 DNA samples extracted from wild boars and pigs' tissues (ASFV positive and negative) in National Veterinary Research Institute in Pulawy (Poland 2018) were tested to demonstrate sensitivity and specificity of the kit. The result of the study displayed 100% sensitivity and 100% specificity in all the samples (spleen, bone marrow, lung, kidney, lymph node, tonsil, blood) comparing the result of Fernandez-Pinero method (UPL-162 probe). A serial dilution of the ASFV's DNA with known viral titer and a quantified ASFV plasmid were assessed and led to a limit of detection (LOD) of $10^{1.16}$ HA_{50} per mL and 1 copy of nucleic acid per PCR.

2. INTRODUCTION

African swine fever virus (ASFV) is a large double stranded DNA virus with lipoprotein envelop and belongs to a genus Asfivirus and a family *Asfaviridae*. ASFV is highly resistant in the natural environment just like other DNA viruses. ASFV can infect all *Suidae* by biting of the infected tick, direct contact with sick animals or by ingesting by products derived from the infected animals. The characteristic symptoms of ASF is high fever, loss of appetite, hemorrhages in skin and internal organs, and high mortality in pigs. ASF is also one of the economically important diseases and has become a real threat in eastern Europe and China. In this situation, many countries near by China were highly concerned about the risk of transmission and the fast and exact diagnosis of ASF is of importance. In this study, the performance of ASFV qPCR kit was evaluated by testing standard panel and field samples of Poland in 2018.

3. MATERIALS and METHODS

VDx® ASFV qPCR Kit is a commercial kit for detection of viral DNA of ASFV by real time PCR method and this kit can measure simultaneously the p72 gene of ASFV and an exogenous Internal process control (IPC) quantatively by using TaqMan probe. The viral DNAs were extracted from 60 test samples (blood, serum and tissue homogenates from pigs and isolated virus) by using QIAmp DNA Mini Kit (Qiagen) and real time PCR was conducted by using 7500AB capable of reading Fluorescence Dyes FAM and HEX (or VIC). All of the samples used for the evaluation were previously tested by the real time PCR UPL tests recommended by EURL in Valdeolmos in Spain and by the International Animal Health Organization (OIE). In addition, the compliance of the obtained results with the EY requirements included in the decision of the European Union commission No. 2003/422 EC of May 26, 2003 approving the Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of laboratory tests results to confirm ASF.

4. RESULTS

A set of 6 ASFV strains of different genotypes (I, II, V, VIII, IX, X) isolated in 2016 and 34 ASFV-infected samples extracted from wild boars and pigs' tissue in Poland, 2018 were tested.
All strains and field samples were detected and the result displayed 100% identity between VDx® ASFV qPCR kit and Fernandez-Pinero method (UPL-162 probe Table1).

Table 1. Sensitivity and specificity of VDx® ASFV qPCR kit

Real time PCR UPL		VDx® ASFV qPCR kit		Total
		Positive	Negative	
Positive (n=40)	Virus (n=6)	6	0	40
	Tissues (n=34)	34	0	
Negative (n=20)		0	20	20
Total (n=60)		40	20	60
Sensitivity	**100**	**Specificity**	**100**	

The limit of detection (LOD) of VDx® ASFV qPCR kit was estimated to be 1 copy of nucleic acid per PCR when evaluating a quantified ASFV plasmid. The LOD of VDx® ASFV qPCR kit, evaluated on ASFV's DNA with known viral titer, is estimated to be $10^{1.16}$ HAD_{50} per mL (Table 2).

Table 2. The LOD of VDx® ASFV qPCR kit

Dilution	ASFV plasmid (10^6 copies/mL)	ASFV (Gen II, $10^{6.62}$ HA_{50}/mL)
10^1	Positive	Positive
10^2	Positive	Positive
10^3	Positive	Positive
10^4	Positive	Positive
10^5	Positive	Positive
10^6	Positive	Negative
10^7	Negative	Negative

5. CONCLUSIONS

VDx® ASFV qPCR Kit is a commercial kit for detection of viral DNA of ASFV by real time PCR method and this kit can measure simultaneously the p72 gene of ASFV and an exogenous internal process control (IPC) quantatively.

The sensitivity and specificity, evaluated on different genotypes of ASFV and 54 field samples of Poland in 2018 (positive and negative) displayed 100% sensitivity and 100% specificity.

The LOD of the PCR was 1 copy of synthesized DNA per PCR and the experimental LOD was $10^{1.16}$ HAD_{50} of ASFV genotype II per mL.

VDx® ASFV qPCR Kit could be a useful tool for early detection of ASFV in various materials from infected animals in order to identify the free state of pigs and it's derived materials for trade.

6. REFERENCES

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