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A triplex PCR method as potential DIVA test for African swine fever virus

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Introduction
Although no vaccine against African swine fever virus (ASFV) is still approved for a global use, several live attenuated vaccine (LAVs) prototypes, from the parental LV17/WB/RIE1 strain, have been produced in the H2020-funded VACDIVA project, one of them (LV17/WB/RIE1-?AB) giving very promising experimental results. Besides safety and protection, one relevant requirement for a vaccine to be authorized and commercialized is the availability of reliable associated DIVA tests. This study aims to develop a DIVA molecular method accompanying the ASFV LAV mutant prototypes derived from LV17/WB/RIE1-?A, which are those specifically lacking the viral A gene that is replaced by the incorporation of a reporter gene. To this end, a triplex real-time PCR method was planned.

Methods
Sets of primer pairs and hydrolysis TaqMan probes were designed to target either the deleted (A gene) or inserted (reporter gene) genome regions. Additionally, previously designed primers and probe for ASFV VP72-coding gene detection (1,2), which are recommended and widely used for the routine ASF diagnostics (2), were incorporated into the multiplex real-time PCR to act as control of ASFV presence. Finally, each probe was labelled with a different reporter fluorochrome allowing the differential detection of the three target genes in a triplex reaction.
A quantified synthetic DNA standard containing the three amplified fragments was used to establish the analytical sensitivity of the triplex PCR. A panel of blood samples (n>150), collected from in vivo studies testing several LV17/WB/RIE1-?A-derived mutant prototypes, was analysed to evaluate the performance of the developed DIVA test. Briefly, DNA extraction was followed by the triplex DIVA PCR assay that was run in parallel to the routine ASFV diagnostic PCR test (1,2).

Results
Optimal PCR conditions were established to obtain a similar sensitivity for each PCR target gene. Triplicates of serial dilutions of the quantified DNA standard were assayed and the detection limit of the triplex DIVA PCR was estimated to be <10 DNA copies for the three target fragments (ASFV-A deleted, reporter, and ASFV-VP72 genes).
As expected, blood samples taken before vaccination with any LV17/WB/RIE1-?A-derived prototype reported negative PCR result. After immunization, results of the DIVA PCR for the ASFV-VP72 and reporter genes were in agreement with the parallel results of routine VP72 PCR, while samples remained negative for ASFV-A deleted gene. After challenge with virulent ASFV strain, DIVA PCR allowed the discrimination of the presence of the vaccine prototype and the challenge virus showing some discordant results for samples with Ct>36.

Conclusions
The developed triplex real-time PCR assay resulted specific and highly sensitive for the reliable discrimination between the produced LAV prototypes lacking gene A and field ASFV, assuring that any residual viremia, in case it is produced, will be detected. In summary, the presented PCR method enables the immediate DIVA principle application if a vaccination campaign is implemented using LV17/WB/RIE1-?AB or other derived mutant as vaccine.

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