ABSTRACT

African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease that can cause significant economic losses. The disease is widely endemic in parts of Africa, Southern Europe, and increasingly becoming a threat in Eastern Europe. As there is still no vaccine or treatment against the disease, monitoring and controlling of the disease by means of diagnostic tests is the only way to control the disease and is of utmost importance. A new duplex real-time PCR kit that targets the p72 gene and an internal control has been developed and its performance for diagnosis of ASFV has been assessed. In order to demonstrate the sensitivity and specificity of the new LSI VetMAX™ African Swine Fever virus detection kit, different internal and field studies including animal infection experiments were carried out (INIA, Spain; CVI, Netherlands; Germany). 1600 negative samples from ASFV free regions (Germany & Spain) and 33 different pathogens were tested to demonstrate specificity of the assay. About 100 ASFV positive samples from Africa and Europe were also tested. Results of the ASFV kit showed 100% sensitivity in all tested sample materials (blood, serum and tissues) and 100% specificity. No cross-reaction was observed with other pathogens and a serial dilution of the ASFV target sequence led to a limited decrease in the cts copies per PCR reaction. The experimental LOD was 510^3 cts per mL in serum and 1x10^5 cts per mL in blood.

RESULTS

The LSI VetMAX™ African Swine Fever virus detection kit fulfills all the validation criteria of PCR characteristics and method, as required by the NF U47-602-2 standard. The kit was assessed on a panel of DNA isolates from 58 ASF positive samples representing four major ASFV strains originating from different ASFV free areas in Spain (INIA), Portugal (CVI), and Tanzania (INIA). As indicated in the table above, the kit showed 100% sensitivity for the strains tested. The kit was evaluated on a panel of 33 pathogens close to ASFV, including bovine, porcine, and human pathogens. No cross-reaction was observed with other pathogens.

CONCLUSIONS

The LSI VetMAX™ African Swine Fever virus detection kit is a real-time PCR kit allowing the simultaneous detection of ASFV and an exogenous positive control in blood, serum, and tissues samples. The kit fulfills all the validation criteria for PCR characteristics and method as required by the NF U47-602-2 standard. ("Requirements and recommendations for the development and validation of ITR-PCR in Animal Health").

The kit is highly sensitive and specific for the detection of both nucleic acids in the same reaction. The kit detects about 100% of positive ASFV samples/strains and about 100% of negative samples showed 100% specificity on tissues and 100% specificity on blood and serum. ASFV has significant economic impact and high mortality rate. The recent outbreaks of ASFV close to EU borders calls for a sensitive, reliable and specific real-time PCR kit as the one described in this work.

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REFERENCES

• NF U 47-602– Animal health analysis methods – PCR Part 2: Requirements and recommendations for the development and the validation of PCR.
• (http://www.anfr.org)

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Development and validation of African Swine Fever Real-time PCR kit

Figure 1. Disease Distribution maps - 2014 and 2015 (OE)