GARA Scientific Meeting | Rome, 28-30 April 2025

Laboratory Validation Study Of Real-Time PCR Assays For The Detection Of African Swine Fever Virus In Faecal Samples

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INTRODUCTION

African swine fever (ASF) has significant impacts on pig industry worldwide, amplified by the absence of effective and authorized vaccines and antiviral treatments, making a prompt and reliable diagnosis of paramount importance. Laboratory diagnosis targets virus-rich tissues, such as lymph nodes, bone marrow and spleen. Recent studies proposed non-invasive sampling of oral-nasal swabs, rectal swabs and faeces for a faster diagnosis, all lacking data on test performances.

AIM OF THE STUDY: Validation of two WOAH-recommended ASFV Real-Time PCR assays and a commercial amplification kit, based on WOAH guidelines, on swine faeces, providing analytical performances.

MATERIALS AND METHODS

Analytical specificity (ASp) was tested: (1) *in silico* using BLAST software online; (2) *in vitro* by testing swine major enteric pathogens *Salmonella enterica* serovar Derby and serovar London, *Salmonella typhimurium* var. monofasica, Enterotoxigenic *E. coli* (ETEC), *Brachyspira pilosicoli, Lawsonia intracellularis,* HEV, PEDV, TGEV.

Analytical sensitivity (ASe) was assessed by determining the Limit of Detection (LOD) using a quantified highly virulent genotype II Armenia/2007, produced on PBMC culture, heat-inactivated at 70°C x 30 min and ten-fold diluted in Vero cell culture lysate for internal control detection (β-actine).

Each dilution was extracted in triplicate using three manual DNA extraction kits:

- **High Pure PCR Template Preparation Kit** (Roche)
- **QIAamp® Fast DNA Stool Mini Kit** (Qiagen), stool-specific
- **AllPrep PowerViral DNA/RNA Kit** (Qiagen), stool-specific

DNA extracts were amplified through Real-Time PCR, following both **King** and **UPL WOAH protocols** and using **ID Gene™ African Swine Fever Duplex Kit** (Innovative Diagnostics, France) (Fig. 1).

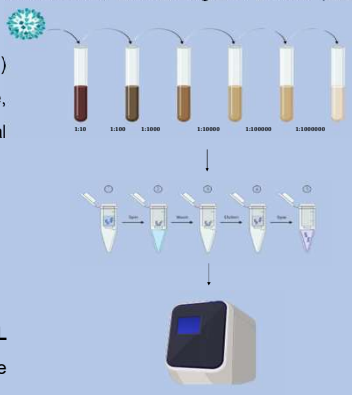

Figure 1. Workflow for ASe assessment. Created with BioRender.com

Interference on ASe was analysed by spiking the stock reference ASFV into a faecal suspension, after mixing ~1 gr of ASFV-free pig faeces and four volumes of Minimum Essential Medium (MEM), enriched with 1% antibiotics.

RESULTS

Upon ASp assessment, primers and probes from the King and the UPL protocols showed 100% identity and query coverage with recent Genbank sequences of the target *B646L* gene. *In vitro*, no amplification curve was observed by Real-Time PCR, thus confirming the absence of cross-reactivity with other porcine pathogens. The higher ASe (50 - $5 \cdot 10^{-1}$ HAD50/ml) was detected using the Roche kit in combination with King and ID GENE™, respectively, but not with UPL ($5 \cdot 10^{2}$ HAD50/ml). The ASe decreased of 1 Log testing faeces with Roche kit under King amplification protocol, and the ID GENE™ kit showed the lowest Cq values compared to other methods. The ASe was further reduced of 1 Log with the Mini Stool kit under King amplification protocol (Fig. 2).

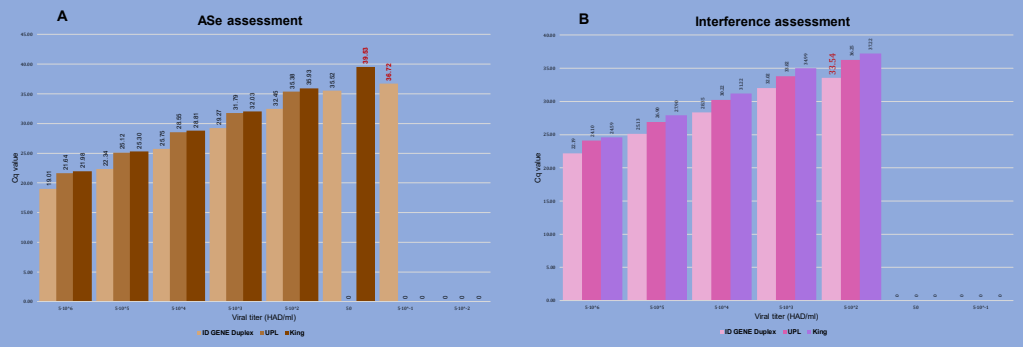

Figure 2. Bar plots of Cq mean values in function of virus titer (HAD/mL) showing performance data of the three amplification protocols upon (A) ASe and (B) interference assessment.

CONCLUSION

This study confirmed the robustness of the WOAH-recommended Real-Time PCR protocols – already validated at the National Reference Laboratory (NRL) for *Pestivirus* and *Asfivirus* on target tissue samples – with a complex matrix as faeces and showed the ID GENE™ provided comparable results to the King protocol. Validation tests are on-going with automated extraction methods. Further diagnostic sensitivity assessment is necessary to provide robust data on the inclusion of faeces and other non-invasive samples in ASFV diagnostic algorithm.

References: Davies *et al.* (2017); de Carvalho Ferreira *et al.* (2014); Fernández-Pinero *et al.* (2013); Flannery *et al.* (2020); King *et al.* (2003).

We wish to acknowledge the IZSPLV (Virology Laboratory) which made this study possible, within the project RC IZSPLV 22C05 CUP J19I22001140001, funded by the Italian Ministry of Health.

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