EVALUATION OF THE VETMAX™-GOLD AIV DETECTION KIT AND VETMAX™-GOLD SIV DETECTION KIT FOR DETECTION OF AVIAN AND SWINE INFLUENZA VIRUSES

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Introduction

Real-time reverse-transcription polymerase chain reaction (RRT-PCR) assays play an important role in the rapid identification of avian and swine influenza A viruses (AIV and SIV, respectively). The aim of this study was to evaluate the Applied Biosystems™ VetMAX™-Gold AIV Detection Kit and Applied Biosystems™ VetMAX™-Gold SIV Detection Kit from Thermo Fisher Scientific (**Figure 1**) for detection of AIV and SIV in clinical samples, respectively and the performance of the Applied Biosystems™ VetMAX™-Gold SIV Subtyping Kit on selected strains.

Materials and Methods

Parallel testing of the VetMAX-Gold AIV Detection Kit and the matrix (M)-gene RRT-PCR assays (APHA-Nagy and APHA-Spackman) for generic influenza A virus detection (Nagy et al., 2010 and Spackman et al., 2002)) was performed on >230 AIV-positives and AIV-negative material (**Table 1**).

Table 1: Summary of samples used to validate the VetMAX-GOLD AIV Detection Kit

Sample types	Species origin	Submission origin	Number
Oropharyngeal and cloacal swabs	Chickens	2015 UK outbreak of H7N7 low pathogenicity AIV	137
Oropharyngeal and cloacal swabs	Wild birds	2018 H5N6 highly pathogenic AIV in UK "found dead" birds	66
Egg-amplified virus	Various	Range of HxNx AIV subtypes	33
Oropharyngeal and cloacal swabs	Poultry	Known AIV-negative status	88

Figure 1: VetMAX-Gold AIV and SIV Detection Kits

The VetMAX-GOLD SIV Detection Kit was similarly compared with the "perfect match" influenza A virus M-gene RRT-PCR assay (APHA-PM based on Spackman design; Slomka et al., 2010) for generic detection of SIV on positive and negative clinical material from the ongoing surveillance project for influenza in the pig population of Great Britain (Slomka et al., 2010). Parallel testing for subtyping of SIV between the APHA RRT-PCR protocols and the VetMAX-Gold SIV Subtyping Kit was performed on a panel of both European and UK SIV isolates.

Results

Complete concordance between the VetMAX-Gold AIV Detection Kit (TF kit) and the APHA-Nagy assay results in detecting 214 AIV samples (**Table 2**). Both tests demonstrated 100% specificity on a panel of 88 AIV-negative RNA samples.

22 additional RNA samples were positive by the VetMAX-Gold AIV Detection Kit using a Ct value of 36.0 as the positive/negative threshold. For the range of AIV subtypes (HxNx), the VetMAX-Gold AIV Detection Kit was significantly more sensitive than the APHA-Nagy assay. VetMAX-Gold SIV kit (TF kit) was on average 1.5 Ct values lower but was not significantly better than the APHA-PM assay, p=0.124 (**Table 3**).

Full SIV subtyping was obtained by the VetMAX-Gold SIV Subtyping Kit on four European isolates producing a partial subtype by the APHA protocols **(Table 4**). Conversely, the APHA protocol provided a full H3N2 subtyping result for two isolates which were partially subtyped (H3Nx) using the VetMAX-Gold SIV Subtyping Kit.

Table 2: 2 x 2 table comparing the diagnostic sensitivities of the VetMAX-Gold AIV Detection Kit and APHA-Nagy assay

		TF kit +	TF kit -
APHA-Nagy	+	214	0
	-	22	88

Table 3: 2 x 2 table comparing the diagnostic sensitivities of the VetMAX-Gold SIV Kit and APHA-PM assay

		TF kit +	TF kit -
APHA-PM	+	83	6
	-	1	114

Table 4: 2 x 2 table comparing the diagnostic sensitivities of the VetMAX-Gold SIV Subtyping Kit and APHA subtyping protocols

		TF subtyping kit Full	TF subtyping kit Partial	TF subtyping kit -
APHA-subtyping	Full	36	2	0
	Partial	4	0	0
	-	0	1	0

Discussion and Conclusion

- The VetMAX-Gold kits are very promising for detection of AIV, and for detection and subtyping of SIV.
- The VetMAX-Gold AIV Detection Kit was on average more than 2.00 Ct values lower than the APHA-Nagy assay.

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