Development of updated porcine reproductive and respiratory syndrome virus products

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that leads to lower reproductive performance in breeding animals and respiratory disease in pigs. Due to the highly mutating nature of PRRSV and newly circulating strains, a project was launched to update the currently on-market PRRSV products with a new molecular design to provide improved detection of the new variants. The new reagents and controls provide a simpler molecular design, which is based on the Applied Biosystems™ VetMAX™ PRRSV EU & NA 2.0 Kit available in Europe. A new molecular design was constructed after bioinformatically analyzing nearly 1,600 sequences from public and private databases as well as *in vitro* testing of nearly 400 positive field samples. Testing results displayed the ability of the new reagents to generate more accurate calls for PRRSV Type II compared to original PRRSV reagents. The new products have been externally tested by multiple collaborator labs and have generated concordant calls with the original products in 94.6% of samples and more accurate calls for 5.15% of total tested samples. The new reagents and controls significantly simplify the testing workflow by using a 2-step master mix compared to the 4-step workflow used by on-market PRRSV reagents and controls. The updated products run in fast mode and decrease the run time from 1 hour and 45 minutes to 49 minutes. These products are for research use only and are not intended to be used in diagnostic procedures.

Introduction

PRRS is a viral disease that leads to abortions and weak-born piglets, increased mortality in suckling and weaned piglets, and respiratory disease in weaners and finishers. PRRSV belongs to the *Arteriviridae* family within the order *Nidovirales* and contains a single-stranded, positive-sense RNA genome of approximately 15 kilobases. PRRSV is split into two genotypes, PRRSV-1 (Type 1) and PRRSV-2 (Type 2), which only share approximately 50% sequence identity and are highly variable within each type. The PRRSV genome encodes ten open reading frames (ORFs). ORF1a and ORF1b encode nonstructural polyproteins with replicase and polymerase activity. ORFs 2, 3, and 4 encode structural glycoproteins GP2, GP3, and GP4, respectively, which are responsible for the formation of a trimeric complex that for viral entry. ORFs 5, 6, and 7 encode the major structural proteins GP5, matrix (M), and nucleocapsid (N), respectively.

The current VetMAX PRRS 1.0 NA and EU PRRSV Reagents contain primers/probes that enable multiplex, one-step reverse transcription quantitative PCR (RT-qPCR) targeting North American and European PRRSV RNA and Applied Biosystems™ VetMAX™ Xeno™ Control RNA.

The aim of this project was to update the molecular design of the current PRRSV reagents to be more sensitive to the novel mutant sequence of PRRSV. This was achieved by bioinformatic analysis of PRRSV sequences retrieved from private and public databases as well as *in vitro* testing of positive panels retrieved from different locations in the US.

Materials and methods

VetMAX NA and EU PRRSV Reagents (on-market products) and the VetMAX PRRSV EU & NA 2.0 Kit (European-market product) were evaluated *in silico* against nearly 1,600 sequences of whole-genome NA PRRSV publicly available (retrieved from NCBI) as well as sequences from private databases (Figure 1). 319 samples from various locations in the US were tested *in vitro* against the on-market products and the European-market product.

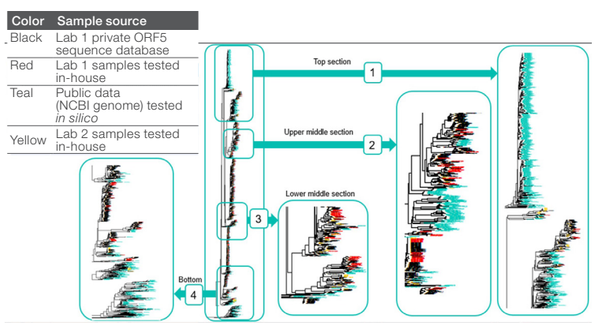

Figure 1. Evaluated PRRSV sequences. The phylogenetic tree of all sequences used for *in silico* and *in vitro* evaluation of VetMAX NA and EU PRRSV Reagents and the VetMAX PRRSV EU & NA 2.0 Kit was constructed using the maximum likelihood method, and local support values at splits were calculated using the Shimodaira-Hasegawa test.

Samples tested *in vitro* were processed using the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700). Different sample types were processed using different workflows and sample input volumes. Extracted PRRSV RNA was tested using the current design and compared to the newly developed products. The new reagents and controls require 2 steps of master mix preparation compared to 4 steps required by on-market products. The new reagents use a fast thermal profile (Table 1) that brings the run time down to 49 minutes compared to the 1 hour and 45 minutes of the on-market PRRSV products.

Table 1. PCR settings and thermal profile used for testing.

PCR settings			
Target	**Reporter dye**	**Quencher**	
PRRSV NA	FAM™	QSY™ NFQ	
PRRSV EU	VIC™		
VetMAX Xeno IPC	Cy®5		
Run mode		Fast	
Passive reference dye		ROX™	
Volume		20 µL	

Thermal profile			
Stage	**Cycles**	**Temperature**	**Time**
1	1	50°C	5 min
2	1	95°C	10 min
3	40	95°C	3 sec
		60°C	30 sec

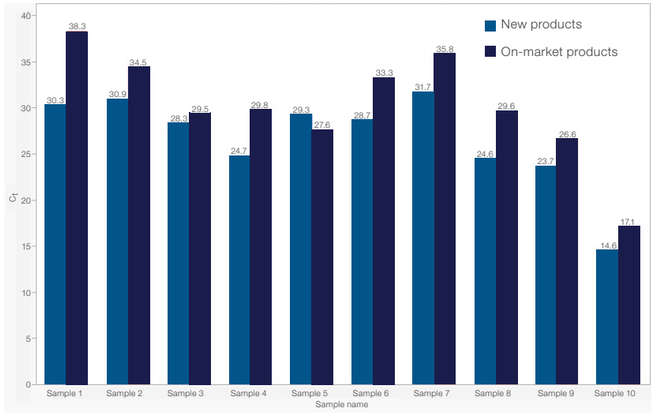

Figure 2. RT-qPCR results comparing new and on-market products for a specific set of samples. The difference in performance between VetMAX PRRSV products currently on the market (dark blue) and the new PRRSV design (blue) plotted in this graph were obtained from collaborator labs. Results obtained with on-market VetMAX PRRSV products were inferior to those obtained with new products. An inconclusive call threshold was a C_t of 37. Extractions were performed using the MagMAX CORE Nucleic Acid Purification Kit and the Thermo Scientific™ KingFisher™ Flex Purification System.

Results

The results of 10 samples that were reported to have lower performance using the on-market PRRSV reagents and controls show that the new molecular design is more efficient, which results in lower C_t values in most cases (Figure 2). The new products generate concordant calls with on-market products while being able to detect the novel mutant sequence of PRRSV.

Testing a large set of samples from different locations in the US as well as different collection time points confirmed the ability of the newly updated reagents and controls to generate concordant calls with on-market PRRSV products (Figure 3).

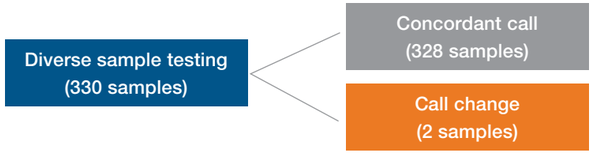

Figure 3. RT-qPCR results comparing new and on-market products for a large diverse sample set. Of the 330 calls, two calls were changed: one from positive to negative (C_t of 35.3 using on-market products to a C_t of 40 using newly developed products) and one from negative to positive (C_t of 38.6 using on-market products to a C_t of 23.9 using newly developed products).

Limit of detection (LOD) of the new products was tested for PRRSV Type 1 and Type 2. The updated reagents and controls can reliably detect down to 10 copies/reaction (data not shown).

Conclusions

The new PRRSV reagents are showing significant improvement over on-market products when testing recent novel PRRSV sequences. The updated molecular design is aimed at achieving similar or better results than the current design, with lower design complexity.

The new PRRSV reagents and controls are more user-friendly as they require fewer master mix preparation steps and run times are significantly shorter (Table 2). The new products were tested by our collaborator labs using large PRRSV-positive samples sets. The product launched in 2021 and replaced the on-market VetMAX PRRSV products.

Table 2. Comparison between on-market and newly developed products.

Criteria	On-market products	New products
Thermal profile	Standard	Fast
Run time	1 hr 45 min	49 min
Reagent preparation steps	4	2
Controls	Requires preparation	Ready to use

Reference

1. Kappes MA, Faaberg KS (2015) PRRSV structure, replication and recombination: origin of phenotype and genotype diversity. *Virology* 479:475-486.

Acknowledgments

We would like to thank Tracy Otterson, Michele Leiferman, and Dr. Stephanie Rossow of University of Minnesota (UMN), and Dr. Michael Zeller, Dr. Phil Gauger, Laura Bradner, and Dr. Karen Harmon of Iowa State University (ISU) for kindly providing PRRSV-positive and PRRSV-negative field samples as well as assisting in building the phylogenetic tree.

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