

# Evaluation of the VetMAX™ PEDV/TGEV/SDCoV Kit, a Multiplex real-time RT-PCR Method for the Detection of Swine Coronaviruses

Lillian Manley<sup>1</sup>, Robert Tebbs<sup>1</sup>, Ivan Leyva Baca<sup>1</sup>, Richard Conrad<sup>1</sup>, Gaëlle Ranaldi<sup>2</sup>  
<sup>1</sup>Thermo Fisher Scientific, Austin, TX, USA, <sup>2</sup>Thermo Fisher Scientific, Lissieu, France

## ABSTRACT

The Applied Biosystems™ VetMAX™ PEDV/TGEV/SDCoV Kit was developed using various types of environmental and related samples obtained from the field, and found to yield high specificity and sensitivity. To examine its performance relative to competitors' solutions, we compared it to 2 other comparable kits prominently used in the market. Environmental, oral fluid, and fecal samples infected with PEDV (N=15), TGEV (N=10), and SDCoV (N=14) were obtained from the field, and negative, positive, and no template controls were included in the testing. RT-qPCR for all three manufacturers' kits were run on the Applied Biosystems™ 7500Fast real-time PCR System according to the manufacturer's recommendations. The VetMAX™ PEDV/TGEV/SDCoV kit consistently showed equivalent or better results to other kits tested, with consistently lower C<sub>T</sub>'s compared to one of the kits and a higher signal versus baseline noise for the other.

## INTRODUCTION

Three coronaviruses – porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine delta coronavirus (SDCoV) – are pathogens of concern for causing gastrointestinal diseases in pigs. All three coronaviruses show similar initial clinical signs, but treatments and remediation are different for each. Tests specific for each of these can advise the growers on what course should be followed, greatly aiding herd management. The VetMAX PEDV/TGEV/SDCoV Kit (A33402) is an assay that detects and differentiates the three coronavirus species in a single reaction mix. This multiplex RT-qPCR assay uses a different fluorescent dye to identify each pathogen target genomic RNA, and includes controls to ensure the assay is working

## Kits and Reagents Used



Figure 1. VetMAX™ Kits and Reagents used

Samples were extracted using the Applied Biosystems™ MagMAX™ CORE™ Nucleic Acid Purification Kit (PN A32700/A32702) on the ThermoFisher™ KingFisher™ Flex with the specified 'Complex Workflow.' 20 negative controls, 2 negative template controls, 2 positive controls, and 39 infected samples were tested in this experiment. All samples were provided and previously screened by veterinary test labs at the University of Minnesota or Iowa State University.

## RESULTS

Applied Biosystems - VetMAX PEDV/TGEV/SDCoV kit		Supplier 1		Supplier 2	
Component	Vol/Rxn	Component	Vol/Rxn	Component	Vol/Rxn
TaqMan Fast Virus 1-Step Master Mix	5 uL	Mix 1 + IC	18 uL	RNA MMx	10 uL
VetMAX PEDV/TGEV/SDCoV Primer/Probe Mix	1 uL	PEDV/TGEV/PDCoV Primers/Probes	2 uL	PEDV/PDCoV RNA Mix	10 uL
Nuclease-Free Water	6 uL	SUBTOTAL	20 uL	SUBTOTAL	20 uL
SUBTOTAL	12 uL	Sample	5 uL	Sample	5 uL
Sample	8 uL	TOTAL	25 uL	TOTAL	25 uL

Real-time RT-PCR protocol for VetMAX PEDV/TGEV/SDCoV			Supplier 1 RT-PCR protocol			Supplier 2 protocol		
temperature	time	No. of cycles	temperature	time	No. of cycles	temperature	time	No. of cycles
48°C	10 min	1	50° C	10 min	1	50° C	15 min	1
95°C	10 min	1	95° C	10 min	1	95° C	1 min	1
95°C	15 sec	40	95° C	15 sec	40	95° C	15 sec	45
60°C	45 sec	40	60° C	45 sec	40	60° C	30 sec	45

Figure 2. Reagents and Cycling Conditions of all 3 kits tested

The VetMAX™ PEDV/TGEV/SDCoV (AB) kit contained a total of 3 frozen components in the kit including master mix, primer probe mix, and nuclease-free water whereas Supplier 1 and 2 only had 2 reagents provided in the kit. Supplier 2 had reagents in which needed to be re-suspended. VetMAX™ PEDV/TGEV/SDCoV kit requires 8 uL of sample whereas Supplier 1 and 2 only require 5 uL of sample. Although all cycling conditions are similar in temperature and length, the VetMAX™ PEDV/TGEV/SDCoV kit as well as Supplier 1 can screen for 3 targets whereas Supplier 2 can only screen for 2.

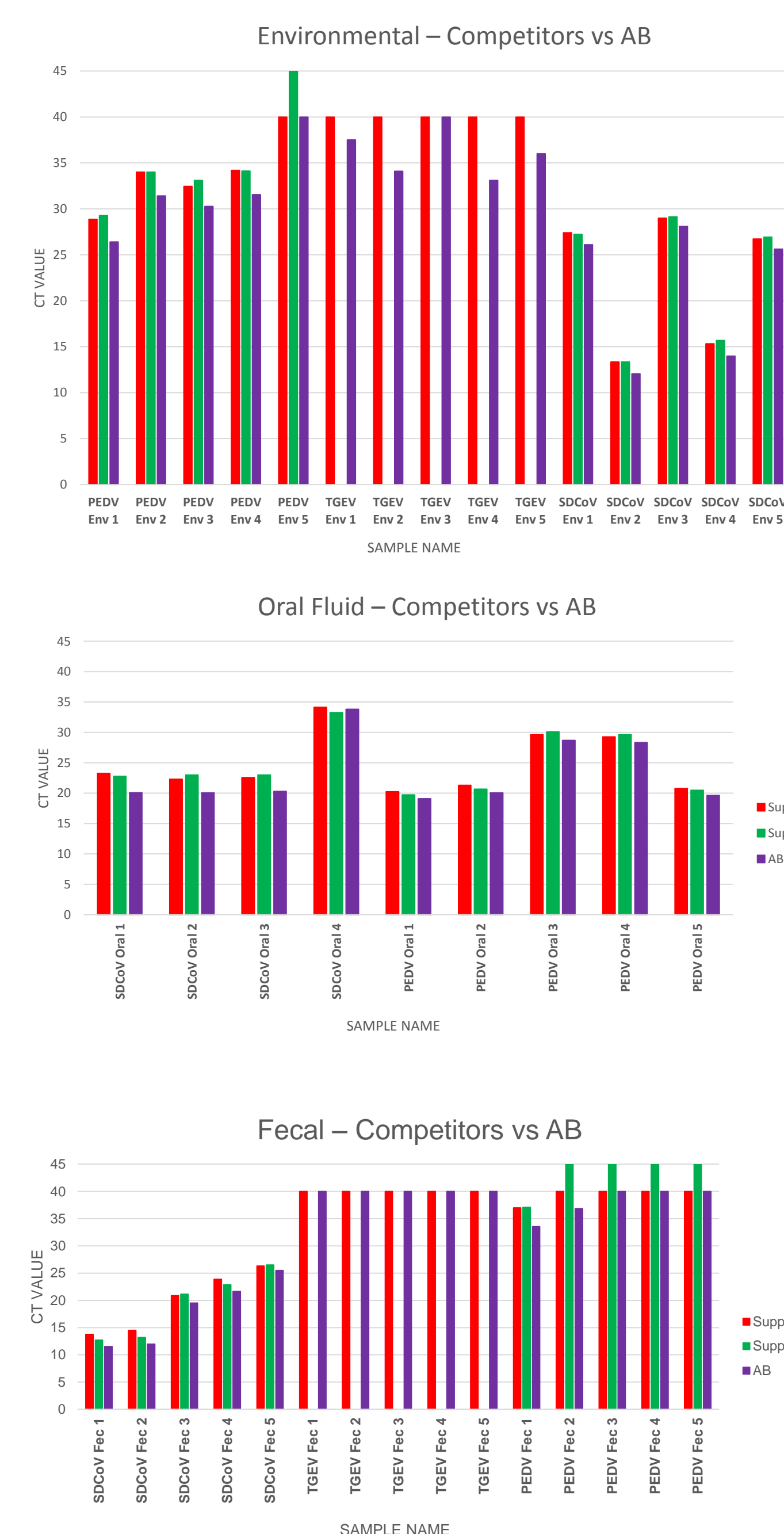


Figure 3. C<sub>T</sub> values and results with automatic threshold

All kits were tested using automatic threshold in order to keep analysis parameters constant. For all sample types (environmental, fecal, and oral fluids), PEDV, TGEV, and SDCoV targeted samples provided the lowest overall C<sub>T</sub> values for the VetMAX™ PEDV/TGEV/SDCoV kit, indicating the VetMAX™ PEDV/TGEV/SDCoV kit is the most sensitive of all 3 kits.

VetMAX™ PEDV/TGEV/SDCoV kit yields the lowest CT values across all 3 sample types of Oral Fluid, Fecal, and Environmental as well as all 3 targets: PEDV, TGEV, and SDCoV.

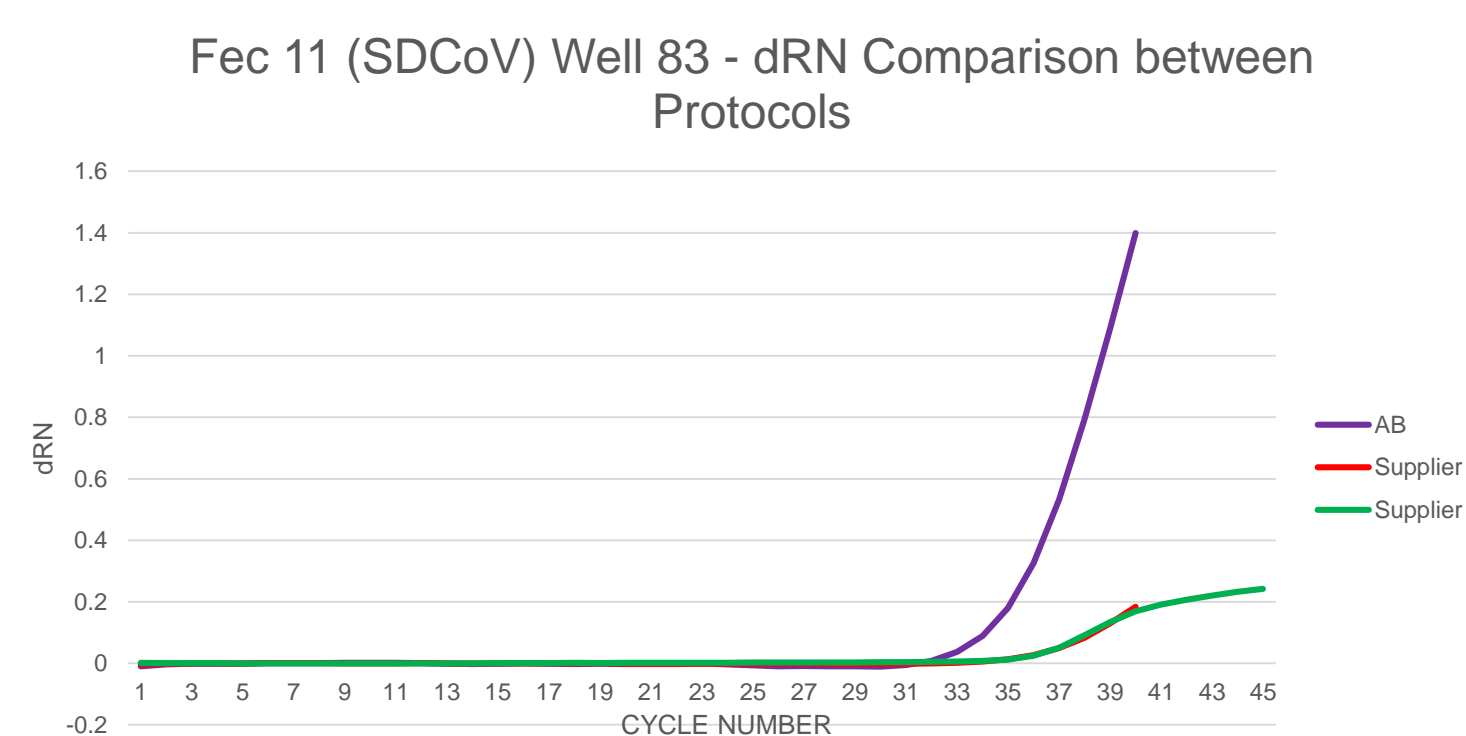
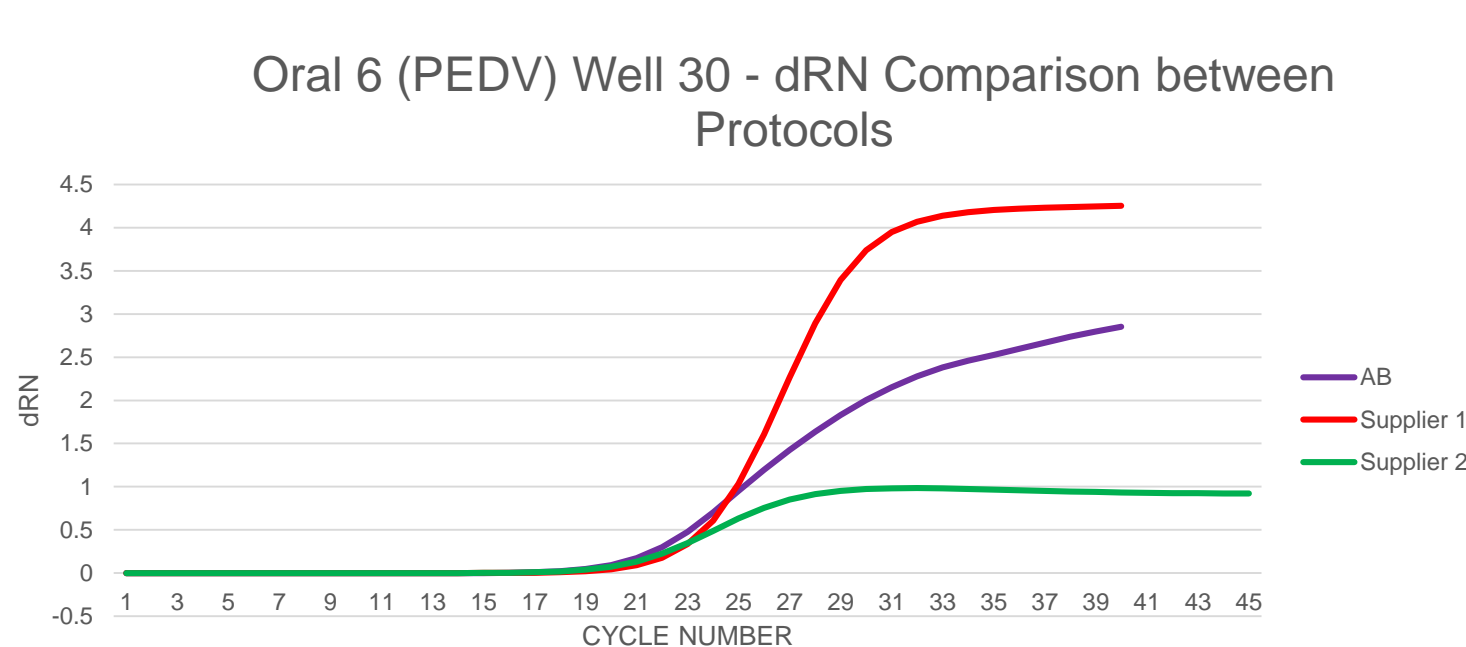
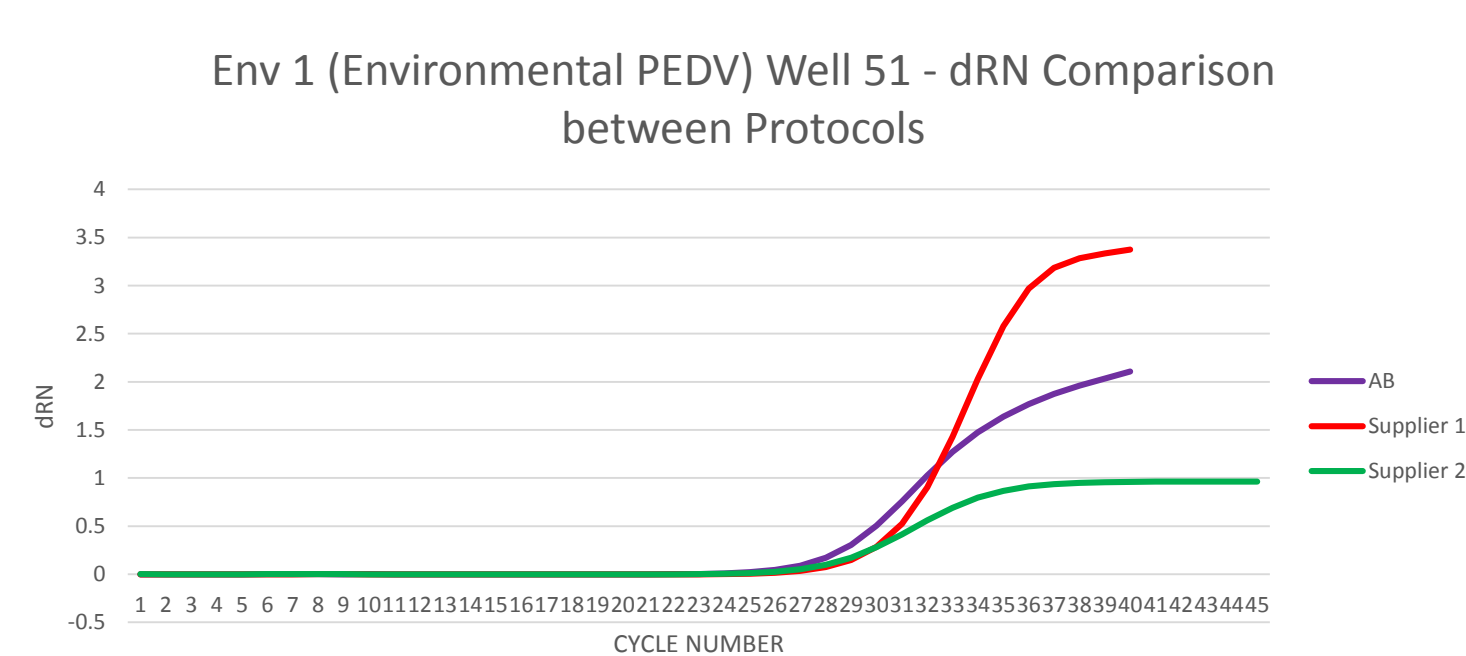
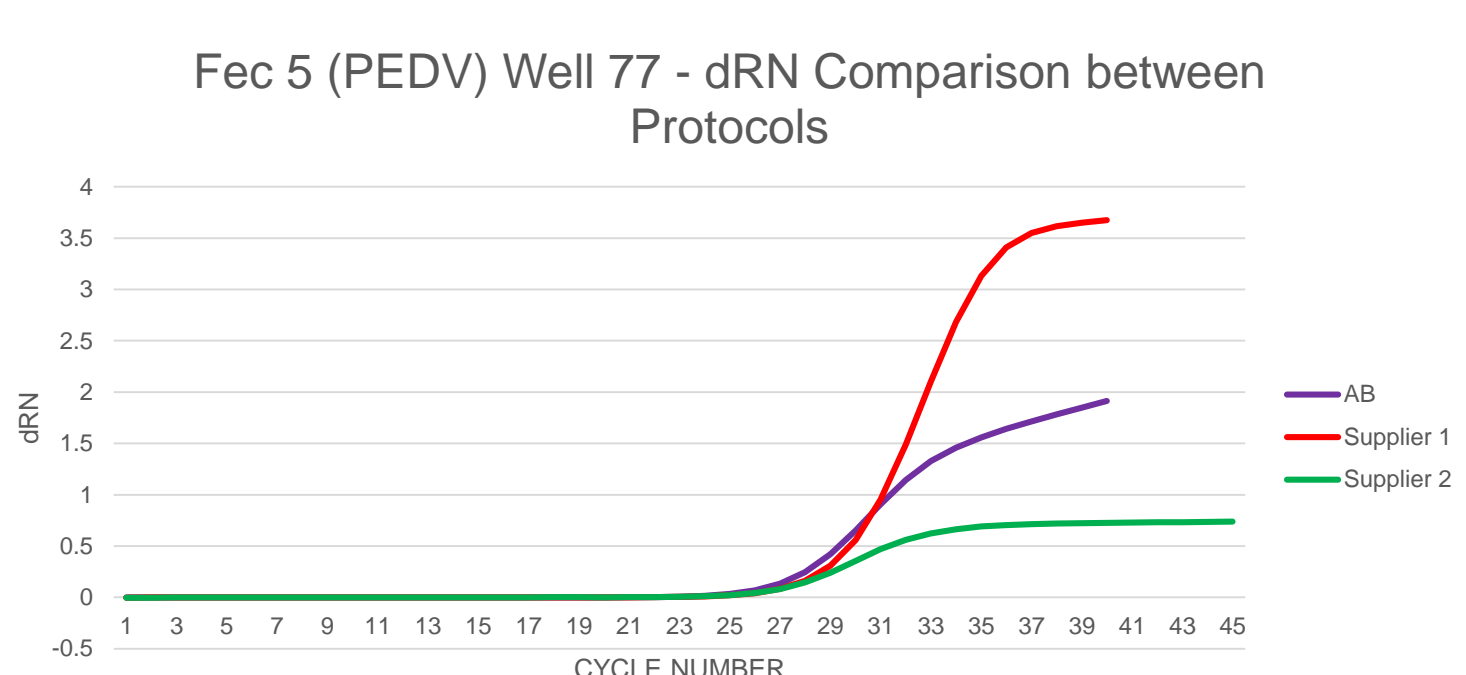
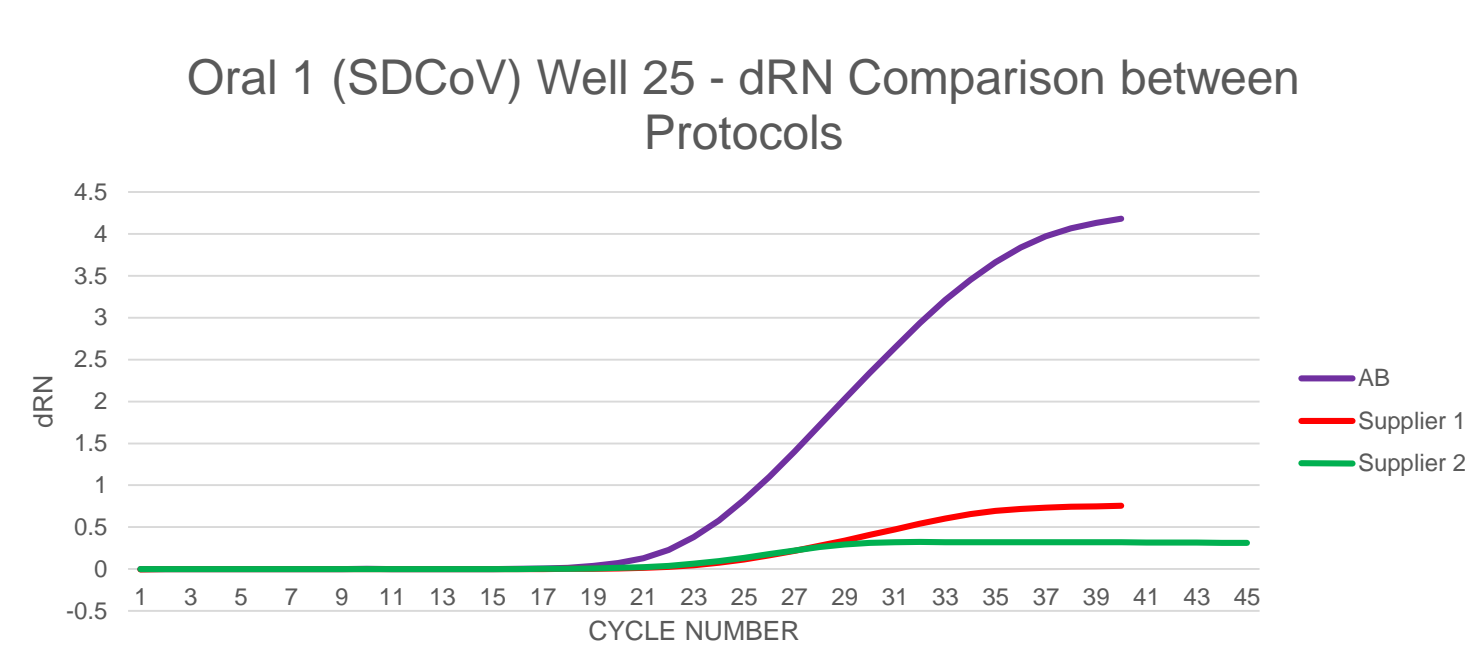
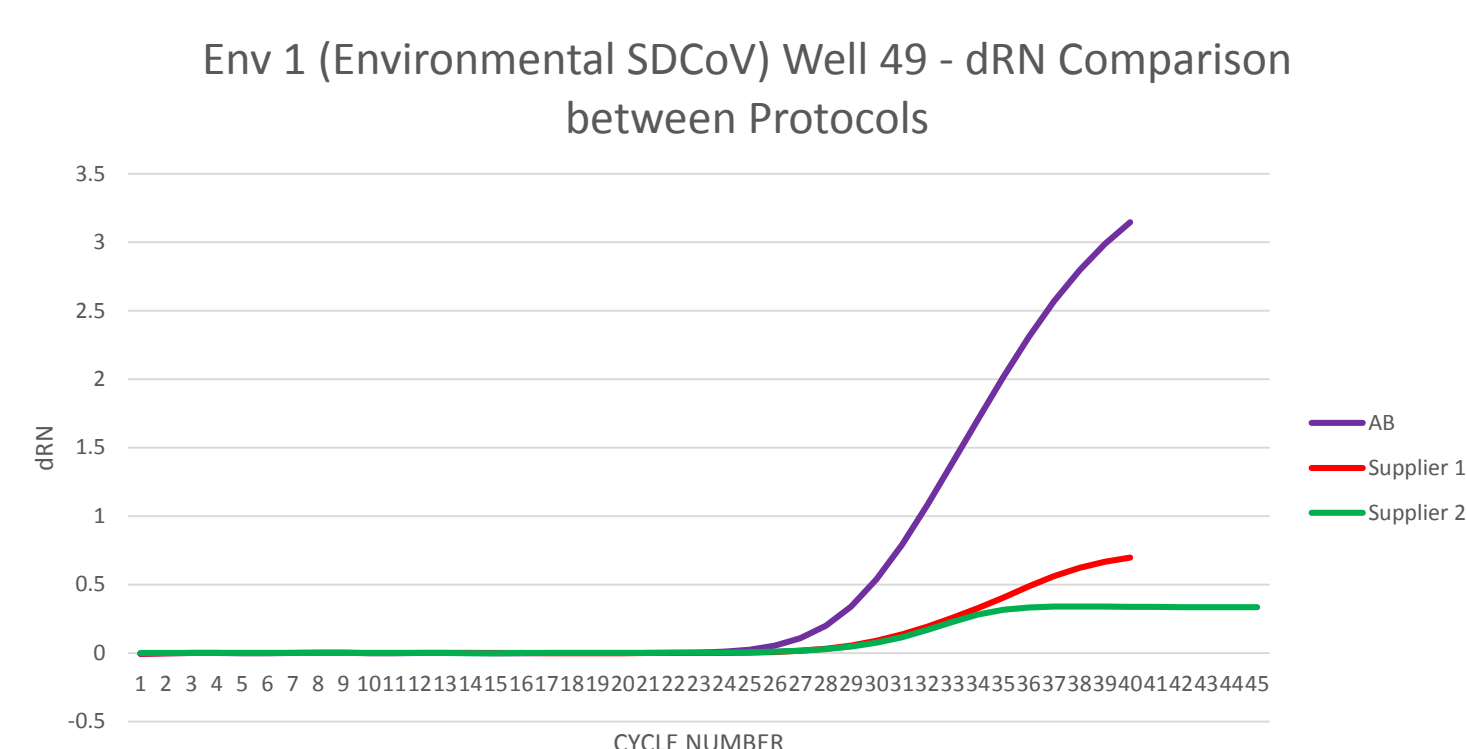


Figure 4. dRN from single wells compared between all three kits

The fluorescence of dyes on the specified targets tends to show up at an earlier cycle number with VetMAX™ PEDV/TGEV/SDCoV. Supplier 1 also shows an early fluorescence signal but it is not as consistent in intensity as VetMAX™ PEDV/TGEV/SDCoV. Supplier 2 shows poor signal to noise with consistently low dRN values. A poor signal to noise with low fluorescence could lead to false positives and poor results. VetMAX™ PEDV/TGEV/SDCoV shows the most consistent fluorescence values leading to good signal to noise values.

	Able to screen for SDCoV, PEDV, and TGEV	Low C <sub>T</sub> value for PEDV	Low C <sub>T</sub> value for TGEV	Low C <sub>T</sub> value for SDCoV	High Fluorescence, good signal to noise
Applied Biosystems	✓	✓	✓	✓	✓
Supplier 1	✓	Shows the weakest C <sub>T</sub>	Shows the weakest C <sub>T</sub>	Shows the weakest C <sub>T</sub>	✓
Supplier 2	✗	✓	N/A	✗	✗

Figure 5. Summary of Target Screen and C<sub>T</sub> (C<sub>q</sub>) Values

The Figure above summarizes the benefits of using Applied Biosystems VetMAX PEDV/TGEV/SDCoV Kit over Supplier 1 and Supplier 2. Overall, it is able to screen for all 3 targets and yields the best Ct values comparatively.

## CONCLUSIONS

VetMAX PEDV/TGEV/SDCoV kit is not only able to screen for all 3 viruses (unlike Supplier 2), but it also shows consistently lower C<sub>T</sub> values for PEDV and TGEV compared to Supplier 1, and has consistently lower C<sub>T</sub> values for SDCoV targets compared to Suppliers 1 and 2. Both the Applied Biosystems™ and Supplier 1 demonstrated a higher fluorescence compared to Supplier 2 which provides good signal to noise ratios and reduces the risk for false positives from baseline drift. The VetMAX PEDV/TGEV/SDCoV Kit allows herd health monitoring for the three viruses even with "dirty" environmental samples. Swine coronavirus pathogens tests are highly requested by laboratories as more and more swine operations are tightening their biosecurity standards to closely monitor for these economically devastating diseases. With this kit we provide laboratories with an efficient tool to help strengthen biosecurity levels on farms.

## REFERENCES

Robben N, Moine S, Tebbs R, Burrell A, Allred A, Swimley M, Hoang Q, Callahan J, Conrad R. Detecting Porcine Coronaviruses PEDV, PDCoV and TGEV by Reverse Transcriptase Real-Time PCR. Poster presented at: 9<sup>th</sup> Annual European Symposium of Porcine Health Management; 2017 May 3-5; Prague, Czech Republic.

## ACKNOWLEDGEMENTS

**Thermo Fisher Scientific R&D Team**  
 Robert Tebbs, Angela Burrell, Michelle Swimley, Quoc Hoang, Ivan Leyva Baca, Rick Conrad

**Thermo Fisher Scientific Team**

Gaëlle Ranaldi, Patrick Buholzer

**Porcine Field Samples**

University of Minnesota, Iowa State University

For Testing of Food and Environmental Samples Only. ©2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.