Comparison Test of RNA Sample using ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix & ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix (Improved Version)

ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix and ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix (Improved Version) are both designed for fast and easy one step real-time PCR reaction set up to amplify any RNA template including mRNA, total RNA and viral sequences. Both master mixes can be used in ONE STEP real-time PCR by removing the reverse transcription step; and both master mixes can achieve excellent results in reaction efficiency, correlation coefficient and slope.

Improved version – **ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix** has few differences:

- Master Mix concentration at **5X**
- Contain Reverse Transcriptase generation III and Hot Start *Taq* generation II
- Contain dNTP/dUTP Mix, RNase Inhibitor and Heat-labile UDG that allow reaction preparation less contamination and leading **to increased performance in sensitivity and specificity**

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Comparison Test

Sample being used to do comparison test:

1. Extracted RNA of Dengue serotype 3 cell culture

   Dengue serotype 3 cell culture from ATCC and in-house, volume 200µl of sample used to extract RNA by using Vivantis GF-1 Viral Nucleic Acid Extraction Kit. Extracted RNA was used for real-time PCR using ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix and ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix.

   All the extracted RNA used did not dilute; 1µl of extracted RNA used straight in real-time PCR reaction.

Components	**Reaction (µl)**
ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix	10
Primer/Probe Mix	1
Dengue extracted RNA	1
Nuclease-free Water	8
Final Volume	**20**

Components	**Reaction (µl)**
ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix	4
Primer/Probe Mix	1
Dengue extracted RNA	1
Nuclease-free Water	14
Final Volume	**20**

Step	**Cycles**	**Temperature**	**Time**
Reverse Transcription	1	55°C	15 mins
Enzyme Activation	1	95°C	30 sec
Denaturation	40	95°C	10 sec
Data Collection*		60°C	30 sec

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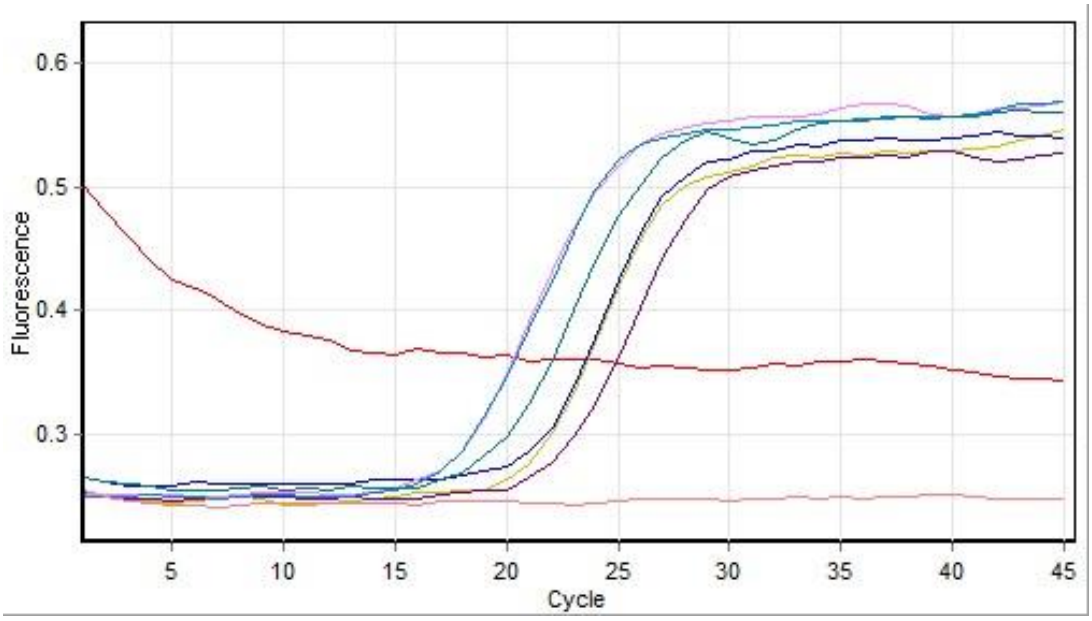
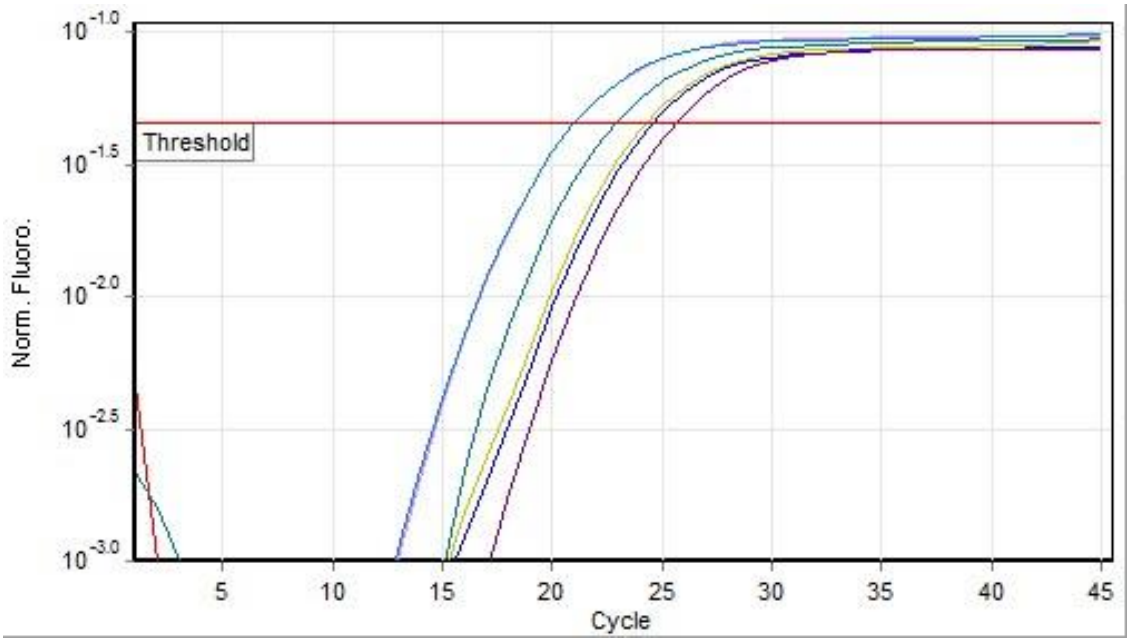
Results

Raw Data For Cycling A.Red (DEN3)



Quantitation data for Cycling A.Red (DEN3)



No.	Color	Name	Type	Ct	Calc Conc (IU/ml)
1		QLMM03 DEN 3	Unknown	24.20	
2		QLMM03 DEN 3	Unknown	24.50	
3		QLMM03 Positive Control ATCC	Positive Control	25.56	
4		QLMM03 DEN 3 NTC	NTC		
5		5X QLMM09 Buffer DEN 3	Unknown	20.95	
6		5X QLMM09 Buffer DEN 3	Unknown	20.98	
7		5X QLMM09 Buffer Positive Control ATCC	Positive Control	22.84	
8		5X QLMM09 Buffer DEN 3 NTC	NTC		

Results showed that **ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix** (5X QLMM09 Buffer) **has CT value earlier** compared to ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix (QLMM03).

2. Extracted RNA of Bacteria culture TOPFA1

Bacteria culture TOPFA1, volume 1ml of sample spin and bacteria pellet is collected and RNA extracted by using Vivantis GF-1 Total RNA Isolation Kit. Extracted RNA was used for real-time PCR using ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix and ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix.

Extracted RNA was diluted to 30ng and 5ng; 1µl of extracted diluted RNA was used in real-time PCR reaction.

Components	**Reaction (µl)**
ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix	10
Primer/Probe Mix	1
Bacteria extracted RNA	1
Nuclease-free Water	8
Final Volume	**20**

Components	**Reaction (µl)**
ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix	4
Primer/Probe Mix	1
Bacteria extracted RNA	1
Nuclease-free Water	14
Final Volume	**20**

Step	**Cycles**	**Temperature**	**Time**
Reverse Transcription	1	55°C	15 mins
Enzyme Activation	1	95°C	30 sec
Denaturation	40	95°C	10 sec
Data Collection*		60°C	30 sec

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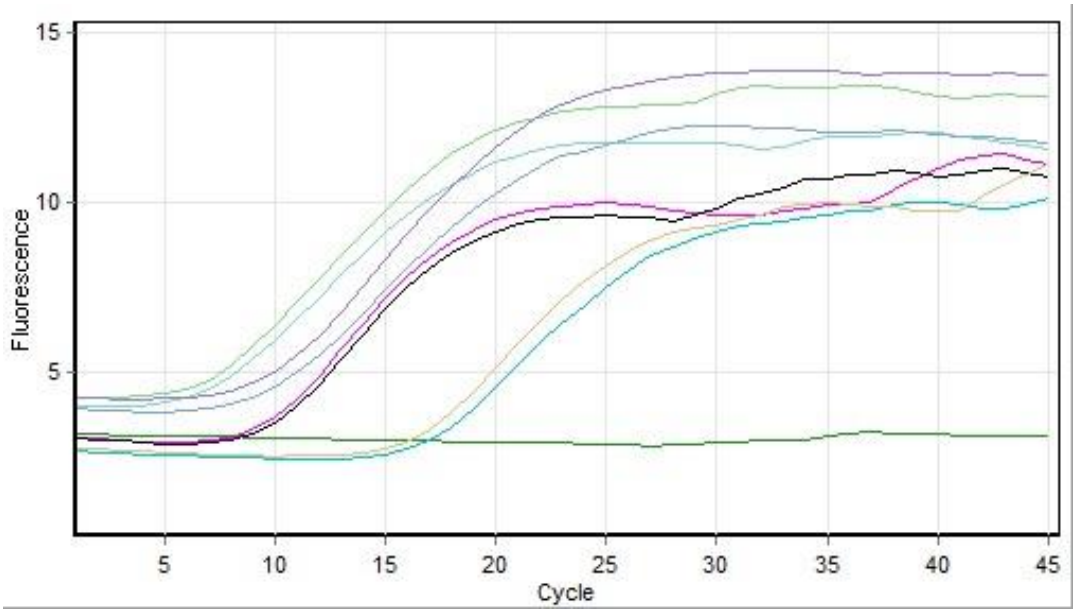
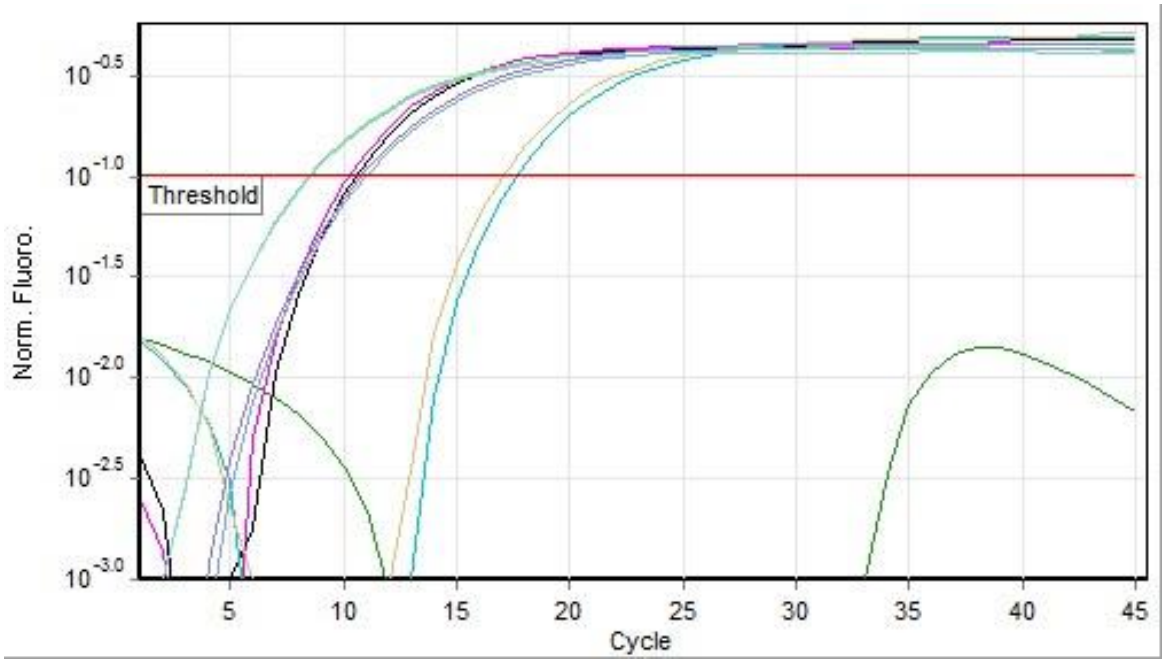
Results

Raw Data For Cycling A.Green (TOPFA1 Bacterial)



Quantitation data for Cycling A.Green (TOPFA1 Bacterial)



No.	Color	Name	Type	Ct	Calc Conc (IU/ml)
1		QLMM03 30ng Bacterial RNA	Unknown	10.21	
2		QLMM03 30ng Bacterial RNA	Unknown	10.53	
3		QLMM03 5ng Bacterial RNA	Unknown	17.60	
4		QLMM03 5ng Bacterial RNA	Unknown	17.02	
5		QLMM03 BacID NTC	Unknown		
6		5X QLMM09 Buffer 30ng Bacterial RNA	Unknown	8.48	
7		5X QLMM09 Buffer 30ng Bacterial RNA	Unknown	8.55	
8		5X QLMM09 Buffer 5ng Bacterial RNA	Unknown	10.96	
9		5X QLMM09 Buffer 5ng Bacterial RNA	Unknown	10.76	
10		5X QLMM09 Buffer BacID NTC	Unknown		

Results showed that **ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix** (5X QLMM09 Buffer) **has CT value earlier** compared to ViPrimePLUS One Step *AtTaq* RT-qPCR Master Mix (QLMM03).

3. Extracted RNA from infected shrimp samples

Shrimp samples with volume 100mg of sample were collected and RNA extracted by using Vivantis GF-1 Tissue Viral Nucleic Acid Extraction Kit. Extracted RNA was used for real-time PCR using Speedy Assay ShrimpCheck Infectious Myonecrosis Virus One-Step RT Real-time PCR Kit (QIMNV01) and ShrimpCheck Yellow Head Virus One-Step RT Real-time PCR Kit (QYHV01). ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix was used to replace 2X OneStep RT-qPremix to do comparison.

Extracted RNA was not diluted, 1µl of extracted RNA was used in real-time PCR reaction.

Components	**Reaction (µl)**
2X One Step RT-qPremix	10
10X Primer/Probe Mix – IMNV & YHV	2
Shrimp extracted RNA	1
Nuclease-free Water	7
Final Volume	**20**

Step	**Cycles**	**Temperature**	**Time**
Reverse Transcription	1	42°C	10 mins
Enzyme Activation	1	95°C	2 mins
Denaturation	40	95°C	15 sec
Data Collection*		60°C	60 sec

Components	**Reaction (µl)**
ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix	4
10X Primer/Probe Mix – IMNV & YHV	2
Sample extracted RNA	1
Nuclease-free Water	13
Final Volume	**20**

Step	**Cycles**	**Temperature**	**Time**
Reverse Transcription	1	55°C	15 mins
Enzyme Activation	1	95°C	30 sec
Denaturation	40	95°C	10 sec
Data Collection*		60°C	30 sec

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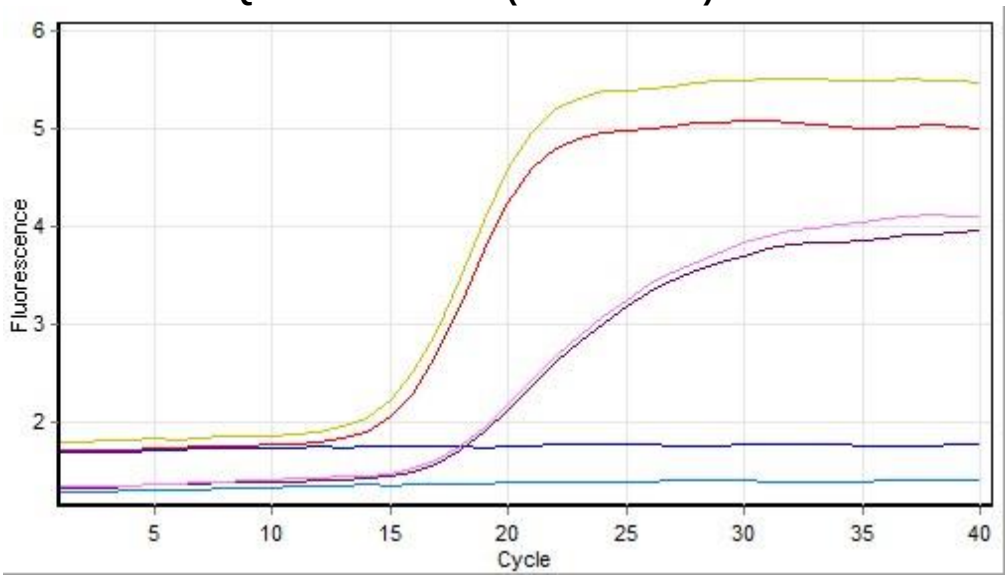
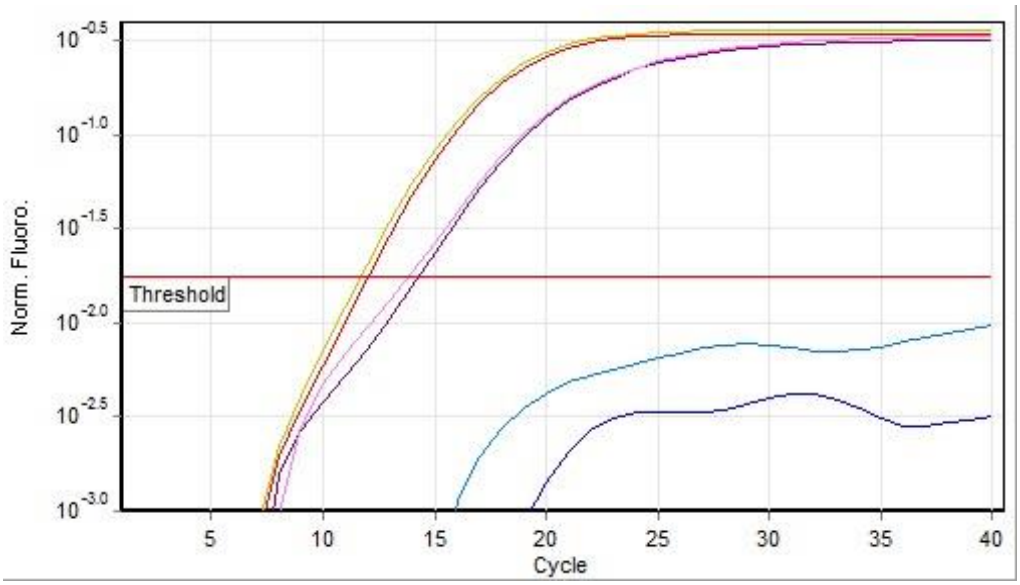
Results

ShrimpCheck IMNV RT-qPCR Kit & ShrimpCheck YHV RT-qPCR Kit using kit master mix

Green FAM Raw Data vs Quantitation Data (YHV & IMNV)





No.	Color	Name	Type	Ct	Calc Conc (Copies)
1		ShrimpCheck YHV + EEC	Unknown	12.04	
2		ShrimpCheck YHV + EEC	Unknown	11.69	
3		ShrimpCheck YHV NTC	Unknown		
4		ShrimpCheck IMNV + EEC	Unknown	14.28	
5		ShrimpCheck IMNV + EEC	Unknown	13.84	
6		ShrimpCheck IMNV NTC	Unknown		

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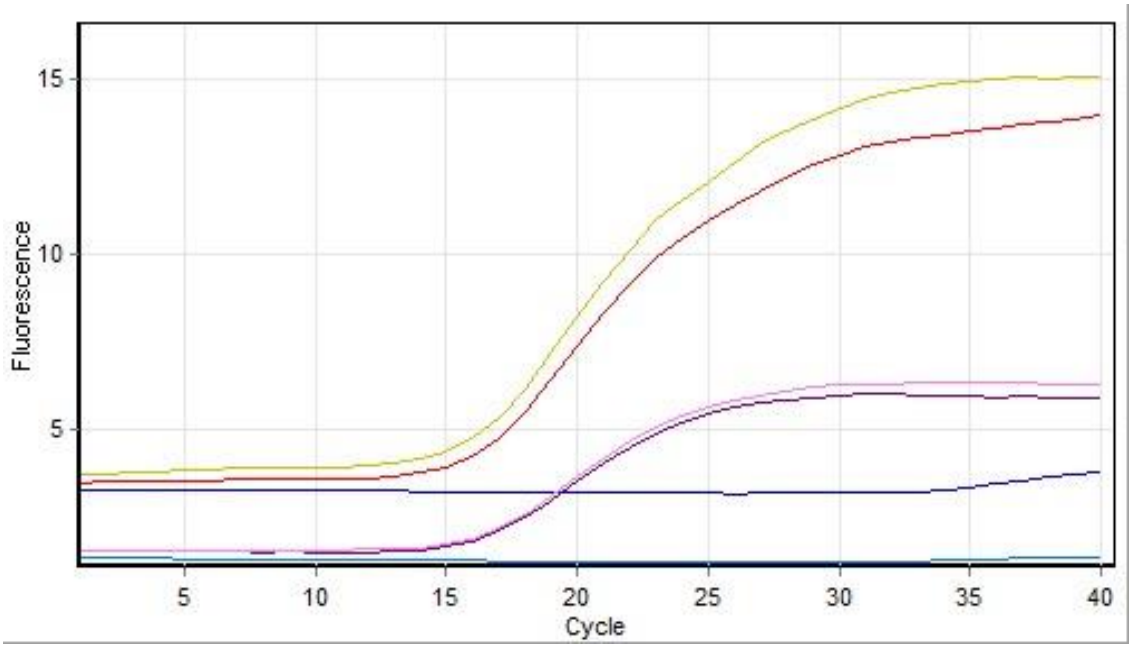
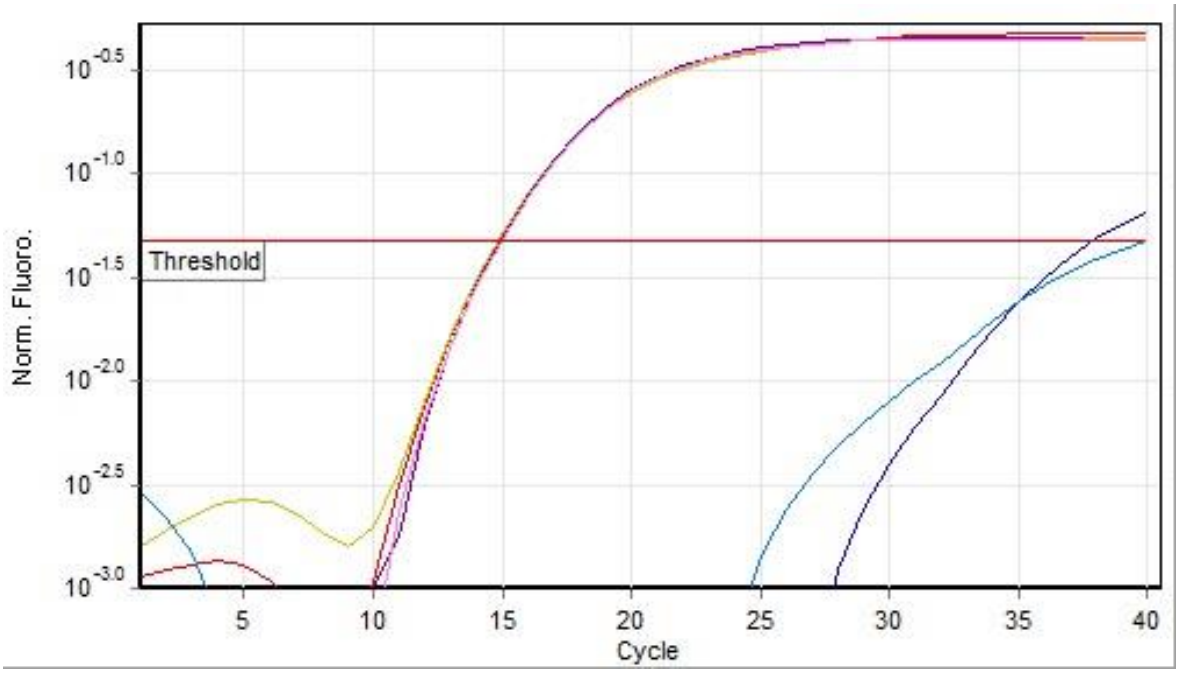
Yellow HEX Raw Data vs Quantitation Data (YHV & IMNV)

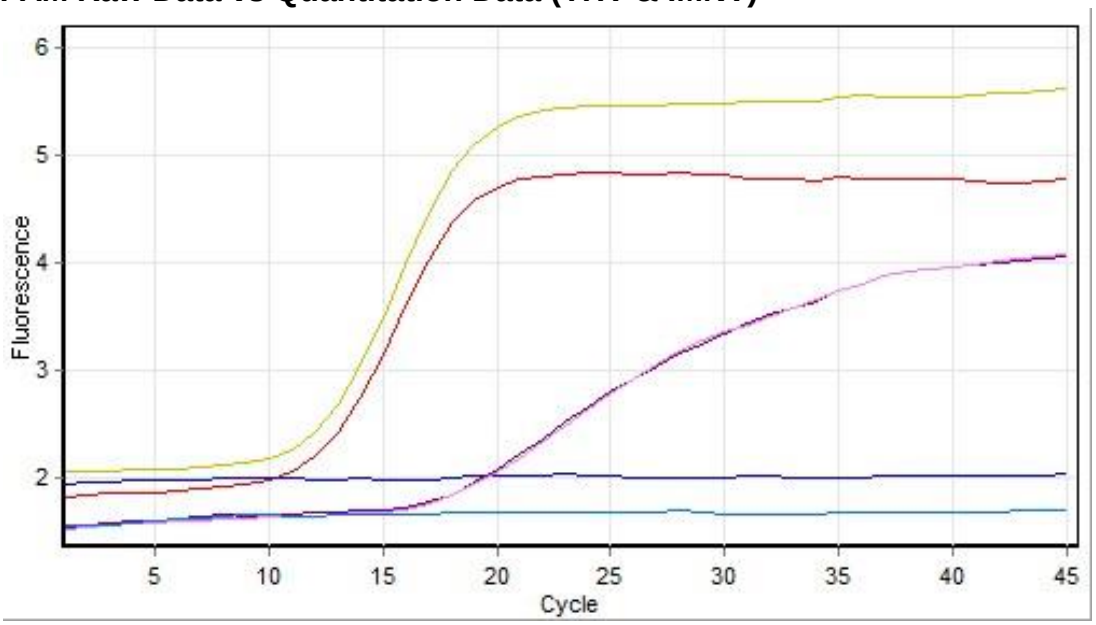


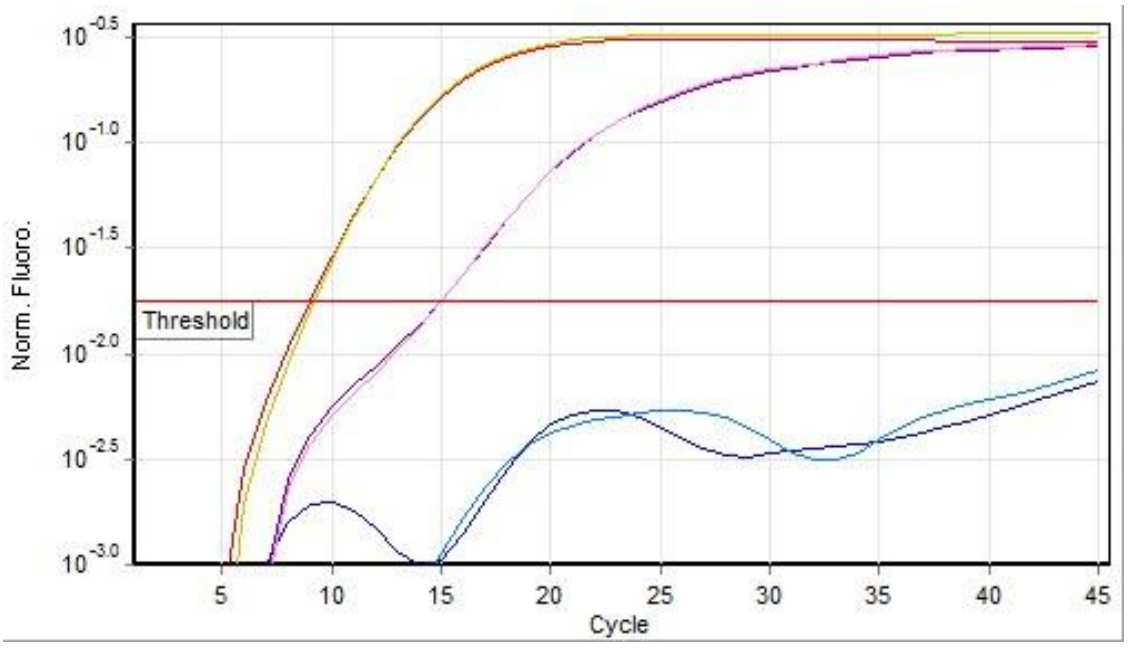


No.	Color	Name	Type	Ct	Calc Conc (Copies)
1		ShrimpCheck YHV + EEC	Unknown	14.83	
2		ShrimpCheck YHV + EEC	Unknown	14.81	
3		ShrimpCheck YHV NTC	Unknown		
4		ShrimpCheck IMNV + EEC	Unknown	14.89	
5		ShrimpCheck IMNV + EEC	Unknown	14.95	
6		ShrimpCheck IMNV NTC	Unknown		

ShrimpCheck IMNV RT-qPCR Kit & ShrimpCheck YHV RT-qPCR Kit using ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix

Green FAM Raw Data vs Quantitation Data (YHV & IMNV)





No.	Color	Name	Type	Ct	Calc Conc (Copies)
1		5X QLMM09 Buffer MM YHV + EEC	Unknown	8.96	
2		5X QLMM09 Buffer MM YHV + EEC	Unknown	9.13	
3		5X QLMM09 Buffer MM YHV NTC	Unknown		
4		5X QLMM09 Buffer MM IMNV + EEC	Unknown	14.96	
5		5X QLMM09 Buffer MM IMNV + EEC	Unknown	15.00	
6		5X QLMM09 Buffer MM IMNV NTC	Unknown		

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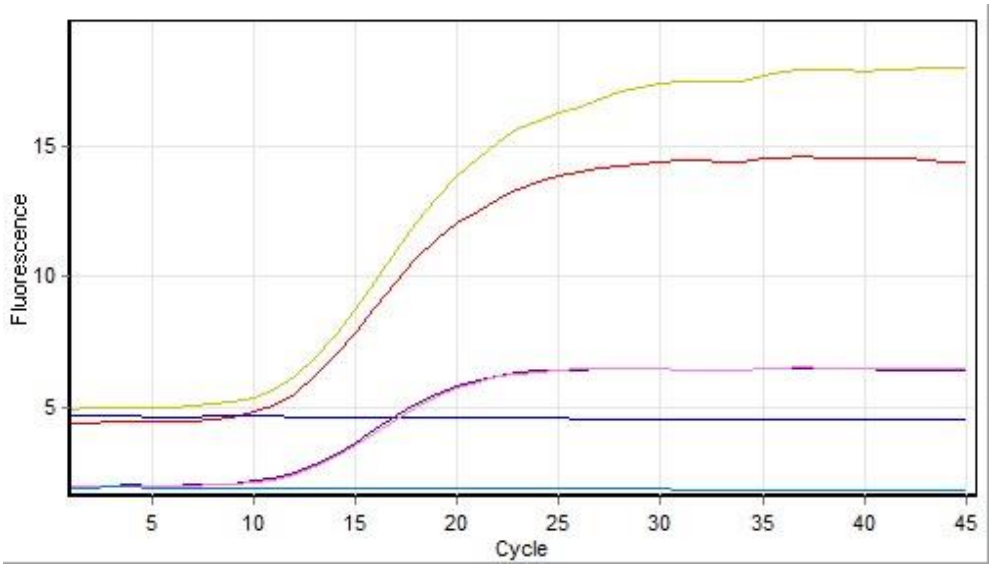
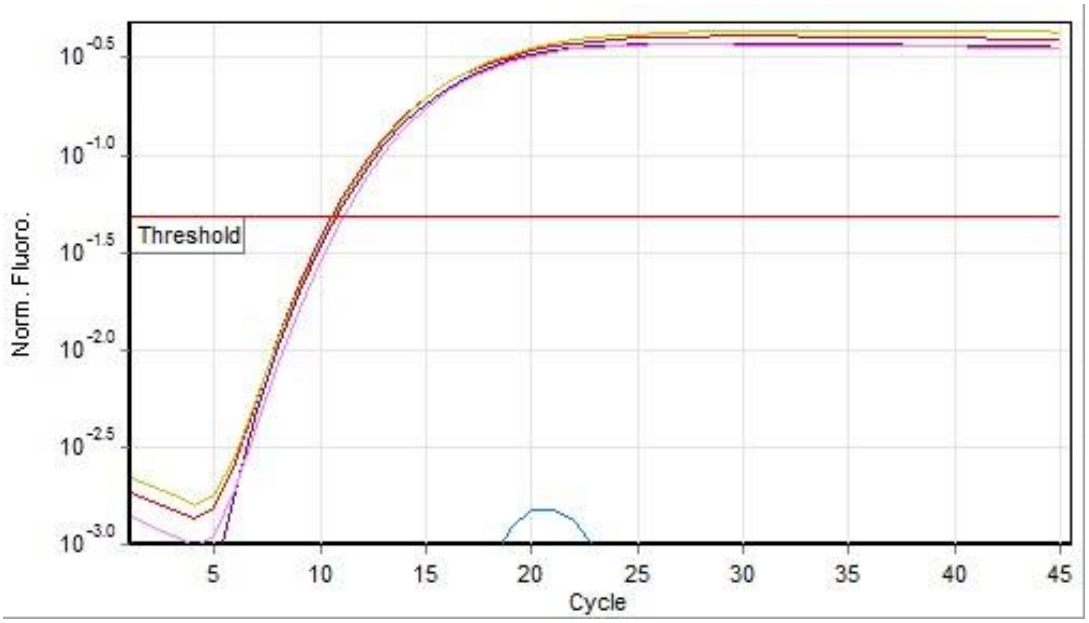
Yellow HEX Raw Data vs Quantitation Data (YHV & IMNV)





No.	Color	Name	Type	Ct	Calc Conc (Copies)
1		5X QLMM09 Buffer MM YHV + EEC	Unknown	10.48	
2		5X QLMM09Buffer MM YHV + EEC	Unknown	10.59	
3		5X QLMM09 Buffer MM YHV NTC	Unknown		
4		5X QLMM09Buffer MM IMNV + EEC	Unknown	10.71	
5		5X QLMM09 Buffer MM IMNV + EEC	Unknown	11.03	
6		5X QLMM09 Buffer MM IMNV NTC	Unknown		

Results showed that ViPrimePLUSHigh 5X One Step RT-qPCR Master Mix used in shrimp testing has CT value earlier – testing increased in sensitivity.