COVID-19 Testing Using Saliva Sample

Saliva sample has suggested to be used as samples for COVID-19 testing in journal paper: https://www.medrxiv.org/content/10.1101/2020.05.13.20100206v1.full.pdf : mucus taken from nose or throat often causes coughing and sneezing, raising the possibility that health care workers become infected themselves; collecting saliva samples can lower the possibility of secondary infections. However, there are strict measure needs to be done by collecting the saliva samples. Closed rooms will need to be prepared to prevent the virus spreading around and disinfect measures in the closed rooms have to be practiced to prevent the risk of contamination to next people.

Sample Collection:

Different methods of saliva collection:

K. : Saliva sample collected after 5 minute of gargling the mouth
F. : Saliva sample collected directly without gargling the mouth
V. : Saliva sample collected at mouth for 3-5 minute without gargling the mouth
S. : Saliva sample collected immediately after gargling the mouth

Saliva samples are spiked with heat inactivated COVID-19 cell culture supernatant.

1. First preserve method:
   Aliquot 200µl of saliva samples added into 200µl of VTM buffer
2. Second preserve method:
   Swab soaked into saliva samples for 5 minutes and soaked into 250µl VTM buffer. Vortex and mix well.

Testing Method for Saliva Samples with Different Collection Method

Saliva samples collected in the falcon tubes without having VTM buffer. Saliva samples kept at 4°C for 3 days.
200µl of saliva samples proceeded to viral RNA extraction by using GF-1 Viral Nucleic Acid Extraction Kit.
Extracted RNA proceeded to real-time PCR testing by checking with human gene detection.

Testing Method for Saliva Samples Spiked with COVID-19 Cell Culture Supernatant

1. 200µl saliva samples from 400µl mixture proceeded to viral RNA extraction.
2. 200µl VTM sample from the swab-VTM tube proceeded to viral RNA extraction.

Extracted RNA proceeded to real-time PCR testing for COVID-19 gene detection.

Limitation:

No SARS-CoV-2 positive-patient / clinical saliva samples available.

Vivantis Technologies Sdn Bhd
200201019726 (587389-D)
A member of Revongen Corporation
Headquarters
Revongen Corporation Center
Level 17, Top Glove Tower, No. 16, Persiaran Setia Dagang,
Setia Alam, Seksyen U13, 40170 Shah Alam,
Selangor Darul Ehsan, Malaysia.

T: +6 03 3359 1166
F: +6 03 3358 0303
E : info@vivantechnologies.com
W: www.vivantechnologies.com

www.qiagen.com

Quantitation Report

Experiment Information

Run Name	Saliva Test [presence of human gene]Run 2020-09-03 (1)
Run Start	9/3/2020 1:43:10 PM
Run Finish	9/3/2020 3:25:29 PM
Operator	Vivantis R&D Team
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.1.49
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain Yellow	5.
Gain Orange	5.
Gain Red	5.
Machine Serial No.	0313306

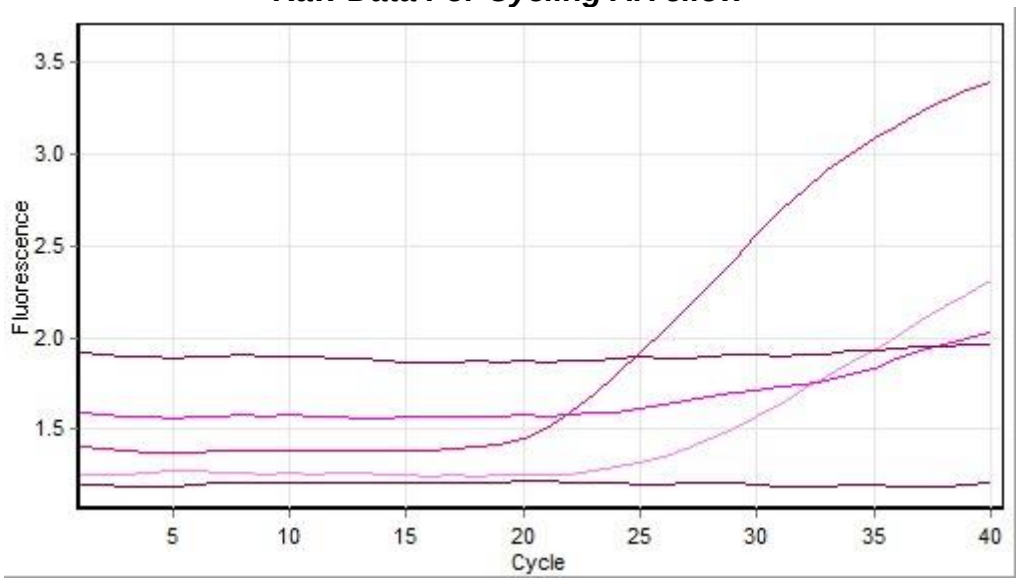
Quantitation Information

Threshold	0.0137
Left Threshold	1.000
Standard Curve Imported	Yes
Standard Curve (1)	conc= 10^(-0.282*CT + 12.894)
Standard Curve (2)	CT = -3.549*log(conc) + 45.761
Reaction efficiency (*)	(* = 10^(-1/m) - 1) 0.91318
Start normalising from cycle	11
Noise Slope Correction	No
No Template Control Threshold	% 0
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

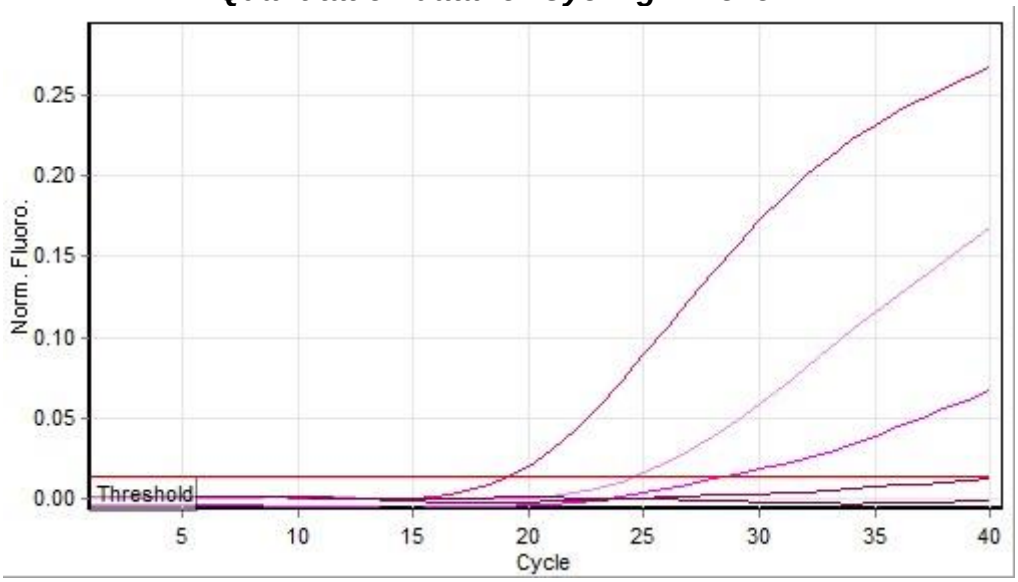
Profile

Cycle	Cycle Point
Hold 1	Hold @ 42°C, 10min 0s
Hold 2	Hold @ 95°C, 2min 0s
Cycling (40 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 60°C, 60s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7],[Yellow][2][2])

Raw Data For Cycling A.Yellow



Quantitation data for Cycling A.Yellow



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
1		Saliva (direct extraction)_K	Unknown	24.63			896,549
2		Saliva (direct extraction)_F	Unknown	28.49			73,576
3		Saliva (direct extraction)_V	Unknown	19.24			29,740,813
4		Saliva (direct extraction)_S	Unknown				
5		NTC	NTC				

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

www.qiagen.com

Quantitation Report

Experiment Information

Run Name	Saliva Test - presence of COVID19 N gene_Run 2020-09-07
Run Start	9/7/2020 11:47:22 AM
Run Finish	9/7/2020 1:29:38 PM
Operator	Vivantis R&D Team
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.1.49
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain Yellow	5.
Gain Orange	5.
Gain Red	5.
Machine Serial No.	0313306

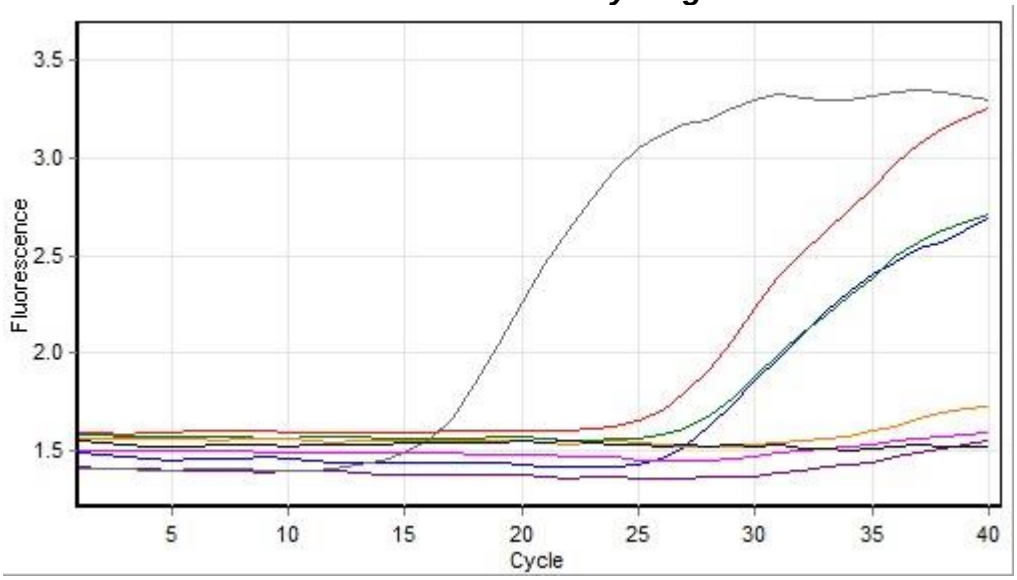
Quantitation Information

Threshold	0.022
Left Threshold	1.000
Standard Curve Imported	Yes
Standard Curve (1)	conc= 10^(-0.280*CT + 13.329)
Standard Curve (2)	CT = -3.565*log(conc) + 47.520
Reaction efficiency (*)	(* = 10^(-1/m) - 1) 0.90763
Start normalising from cycle	11
Noise Slope Correction	No
No Template Control Threshold	% 0
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

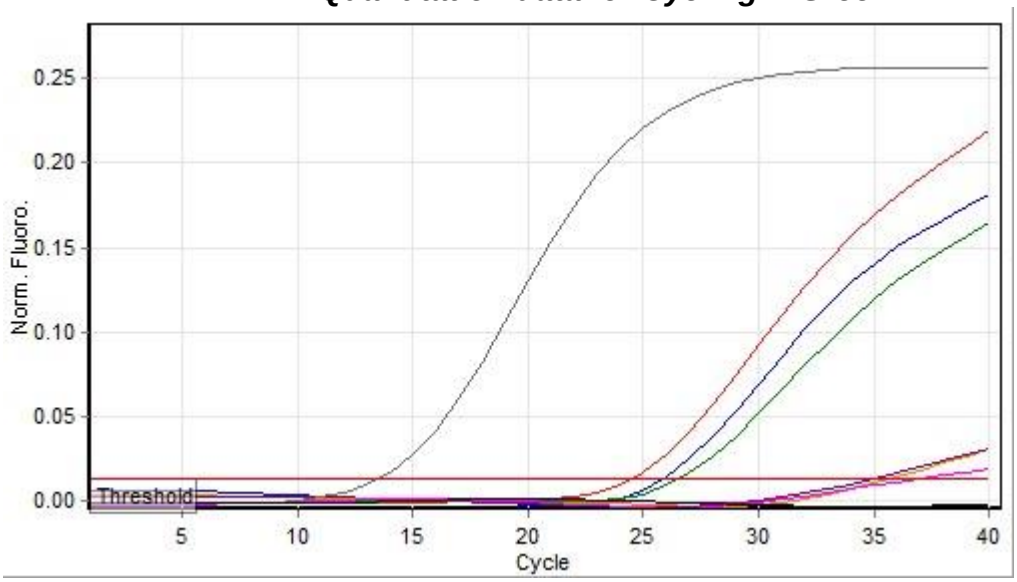
Profile

Cycle	Cycle Point
Hold 1	Hold @ 42°C, 10min 0s
Hold 2	Hold @ 95°C, 2min 0s
Cycling (40 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 60°C, 60s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7],[Yellow][2][2])

Raw Data For Cycling A.Green



Quantitation data for Cycling A.Green



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
1		200uL (Saliva+VTM)_K	Unknown	25.95			381,373
2		200uL (Saliva+VTM)_F	Unknown	26.61			248,448
3		200uL (Saliva+VTM)_V	Unknown	24.53			959,587
4		200uL VTM (swab)_K	Unknown	35.79			644
5		200uL VTM (swab)_F	Unknown	35.13			991
6		200uL VTM (swab)_V	Unknown	37.17			264
7		Pos. Ctrl	Positive Control	13.61			1,148,666,607
8		NTC	NTC				

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

www.qiagen.com

Quantitation Report

Experiment Information

Run Name	Saliva Test - presence of COVID19 E gene_Run 2020-09-07
Run Start	9/7/2020 11:47:22 AM
Run Finish	9/7/2020 1:29:38 PM
Operator	Vivantis R&D Team
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.1.49
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain Yellow	5.
Gain Orange	5.
Gain Red	5.
Machine Serial No.	0313306

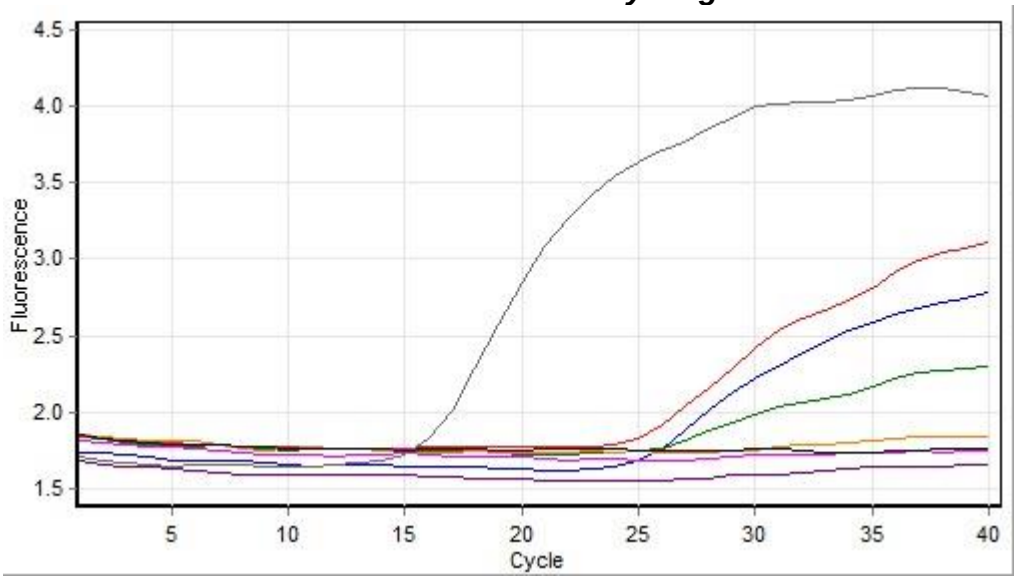
Quantitation Information

Threshold	0.022
Left Threshold	1.000
Standard Curve Imported	Yes
Standard Curve (1)	conc= 10^(-0.280*CT + 13.329)
Standard Curve (2)	CT = -3.565*log(conc) + 47.520
Reaction efficiency (*)	(* = 10^(-1/m) - 1) 0.90763
Start normalising from cycle	11
Noise Slope Correction	No
No Template Control Threshold	% 0
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

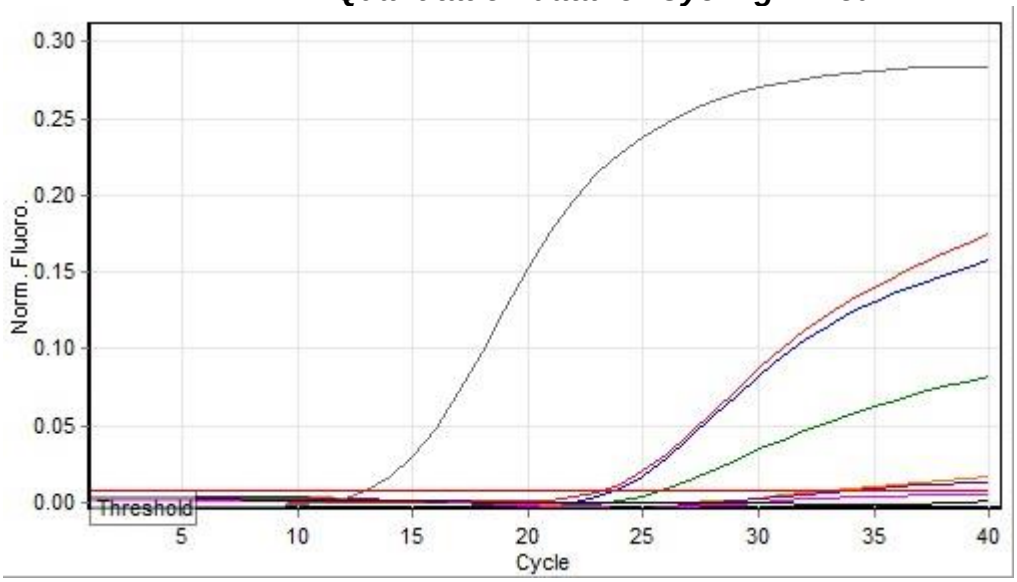
Profile

Cycle	Cycle Point
Hold 1	Hold @ 42°C, 10min 0s
Hold 2	Hold @ 95°C, 2min 0s
Cycling (40 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 60°C, 60s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7],[Yellow][2][2])

Raw Data For Cycling A.Red



Quantitation data for Cycling A.Red



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
1		200uL (Saliva+VTM)_K	Unknown	23.74			1,290,951
2		200uL (Saliva+VTM)_F	Unknown	25.79			317,112
3		200uL (Saliva+VTM)_V	Unknown	23.19			1,887,406
4		200uL VTM (swab)_K	Unknown	32.81			2,527
5		200uL VTM (swab)_F	Unknown	33.30			1,810
6		200uL VTM (swab)_V	Unknown				
7		Pos. Ctrl	Positive Control	12.94			2,185,922,274
8		NTC	NTC				

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

www.qiagen.com

Quantitation Report

Experiment Information

Run Name	Saliva Test - presence of COVID19 RdRP gene_Run 2020-09-07
Run Start	9/7/2020 11:47:22 AM
Run Finish	9/7/2020 1:29:38 PM
Operator	Vivantis R&D Team
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.1.49
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain Yellow	5.
Gain Orange	5.
Gain Red	5.
Machine Serial No.	0313306

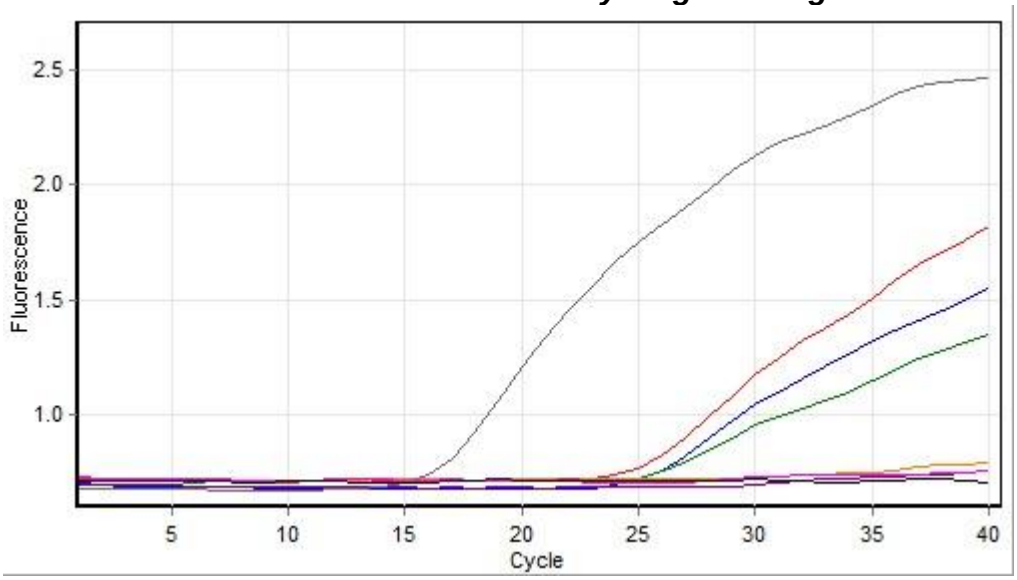
Quantitation Information

Threshold	0.022
Left Threshold	1.000
Standard Curve Imported	Yes
Standard Curve (1)	conc= 10^(-0.280*CT + 13.329)
Standard Curve (2)	CT = -3.565*log(conc) + 47.520
Reaction efficiency (*)	(* = 10^(-1/m) - 1) 0.90763
Start normalising from cycle	11
Noise Slope Correction	No
No Template Control Threshold	% 0
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

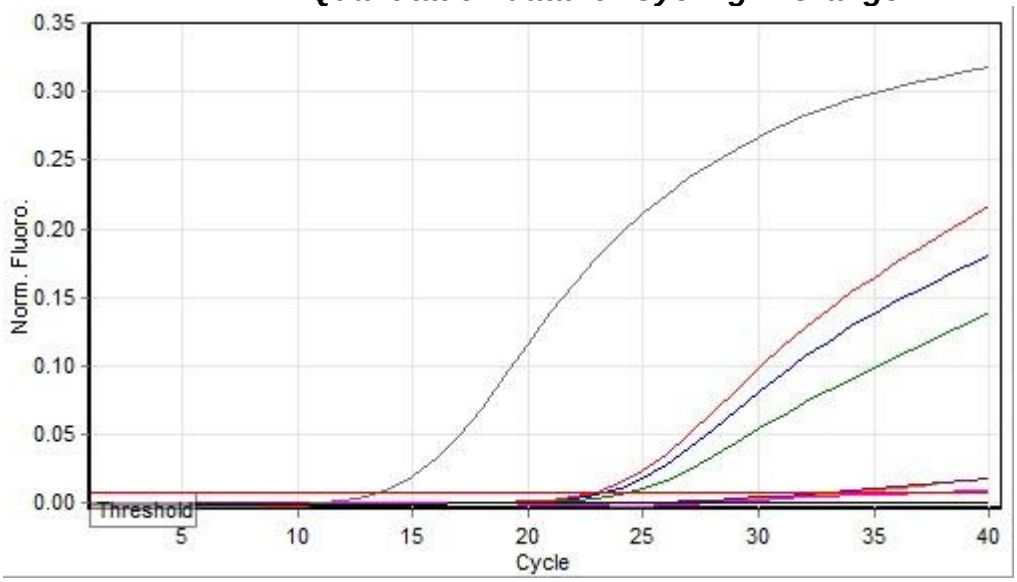
Profile

Cycle	Cycle Point
Hold 1	Hold @ 42°C, 10min 0s
Hold 2	Hold @ 95°C, 2min 0s
Cycling (40 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 60°C, 60s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7],[Yellow][2][2])

Raw Data For Cycling A.Orange



Quantitation data for Cycling A.Orange



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
1		200uL (Saliva+VTM)_K	Unknown	23.32			1,724,883
2		200uL (Saliva+VTM)_F	Unknown	24.38			833,812
3		200uL (Saliva+VTM)_V	Unknown	22.81			2,448,595
4		200uL VTM (swab)_K	Unknown	33.79			1,292
5		200uL VTM (swab)_F	Unknown	32.34			3,506
6		200uL VTM (swab)_V	Unknown	37.14			128
7		Pos. Ctrl	Positive Control	13.51			1,473,261,314
8		NTC	NTC				

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

Conclusion and Recommendation:

GF-1 Viral Nucleic Acid Extraction Kit can be used to extract nucleic acid from saliva sample.

Saliva has to be in mouth for at least 3-5 minutes before collection.
Saliva sample collected immediately after gargling the mouth has no human gene detected, low possibility to detect SARS-CoV-2.
Saliva samples can be kept at 4°C for 4hrs to 8hrs only before proceeding for extraction.

Saliva sample collected from patients advised to mix with VTM buffers to prevent the degradation of COVID-19 virus if any. Saliva sample with VTM buffers can be kept at 4°C within 3 days before proceeding for extraction.
Swab used to soak in saliva sample and soak in VTM buffers has lower viral RNA yield compared to saliva sample mixed directly with VTM buffers.

Prepared by,
Vivantis R&D Team
7th September 2020

Checked by,
Vivantis Lab Manager
8th September 2020

Vivantis Technologies Sdn Bhd
200201019726 (587389-D)
A member of Revongen Corporation
Headquarters
Revongen Corporation Center
Level 17, Top Glove Tower, No. 16, Persiaran Setia Dagang,
Setia Alam, Seksyen U13, 40170 Shah Alam,
Selangor Darul Ehsan, Malaysia.

T: +6 03 3359 1166
F: +6 03 3358 0303
E : info@vivantechnologies.com
W: www.vivantechnologies.com