

# PCRopsis™ Reagents Detect 3.9 viral copies / μL of SARS-CoV-2 in Transport Medium, Saliva, and Dried on Swabs

Francis Buan Hong Lim<sup>1</sup>, Abhignyan Nagesetti<sup>1</sup>, Kevin Moreno<sup>1</sup>, Agustin Galecio<sup>1</sup>, Ian Cheong<sup>1,2,3,4</sup>, Obdulio Piloto<sup>1</sup>

## Affiliations:

<sup>1</sup> Entopsis, Inc., USA

<sup>2</sup> Temasek Life Sciences Laboratory, Singapore

<sup>3</sup> Department of Biological Sciences, National University of Singapore, Singapore

<sup>4</sup> Pathnova Laboratories Pte Ltd, Singapore

Correspondence to: info@entopsis.com

## Abstract:

Extraction-free PCR makes PCR testing more efficient and cost effective. However traditional direct PCR products often fail to detect low levels of virus and thereby have limited utility in the clinical setting. In this report we tested 4 PCRopsis™ direct PCR products on SARS-CoV-2 samples in transport medium, saliva, or dried onto synthetic swabs. These products were tested for their ability to mediate extraction-free amplification at 5 distinct SARS-CoV-2 gene regions. All tested products were able to amplify SARS-CoV-2 when diluted to 3.9 copies / μL (9.75 copies in the PCR mixture) for all tested sample types and 4 of the 5 tested gene regions. Although not tested, it may be possible to detect less than 3.9 copies / μL for some samples types and gene regions.

## Background:

For reference, throat and sputum clinical samples from SARS-CoV-2 infected individuals typically contain 10~10,000 copies / μL at peak infection<sup>1</sup> and nasal swab samples have a mean viral copy number of 1,400 copies / μL.<sup>2</sup> Moreover, respiratory samples from people with mild cases of COVID-19 show a median of over 10 viral copies / μL, while people with severe cases exhibit a median of over 100 viral copies / μL.<sup>3</sup> It was also observed that asymptomatic SARS-CoV-2 infected people tend to have higher viral loads than their symptomatic counterparts.<sup>4</sup> As such, the described PCRopsis™ direct PCR products would have detected the vast majority of cases if used in the clinic.

**Results:**

**A) Reagent RVD with RVD Enhancer (BD™ Universal Transport Medium)**

Copies / $\mu$ L	N1	N2	E	NJ	Orf1a
<b>39,000</b>	21.11	23.59	23.75	28.6	25.3
<b>3,900</b>	24.68	26.79	26.69	31.52	28.3
<b>390</b>	27.9	30.76	29.87	36.75	31.33
<b>39</b>	31.49	34.14	32.56	38.71	34.6
<b>3.9</b>	35.57	38.39	34.92	41.27	36.43

**B) Reagent RVD-RT (BD™ Universal Transport Medium)**

Copies / $\mu$ L	N1	N2	E	NJ	Orf1a
<b>39,000</b>	22.35	25.96	23.3	30.08	28.76
<b>3,900</b>	25.86	26.72	27.39	32.07	30.36
<b>390</b>	28.36	30.66	29.84	36.96	33.12
<b>39</b>	31.32	35.33	31.87	40.12	36.19
<b>3.9</b>	35.27	39.21	34.72		38.36

**Table 1. Reagent RVD with RVD Enhancer and Reagent RVD-RT amplify 3.9 viral copies /  $\mu$ L SARS-CoV-2 spiked into transport medium.** Direct PCR mediated by (A) Reagent RVD with RVD Enhancer (samples processed at 95°C) and (B) Reagent RVD-RT (samples processed at 25°C) resulted in comparable Ct values when 4 SARS-CoV-2 gene regions were amplified. No Ct values were detected for Reagent RVD-RT at 3.9 copies /  $\mu$ L for the NJ gene region. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

**A) Reagent RVD with RVD Enhancer (Human Saliva)**

Copies / $\mu$ L	N1	N2	E	NJ	Orf1a
<b>39,000</b>	23.86	25.81	25.16	28.61	27.96
<b>3,900</b>	25.92	28.84	27.58	31.57	30.32
<b>390</b>	30.35	32.63	30.31	33.91	34.76
<b>39</b>	33.02	36.66	32.89	35.01	
<b>3.9</b>	35.58	38.2	33.81	37.01	

**B) Reagent SRVD (Human Saliva)**

Copies / $\mu$ L	N1	N2	E	NJ	Orf1a
<b>39,000</b>	22.41	24.05	22.53	26.93	25.22
<b>3,900</b>	25.98	28.19	25.95	29.52	28.8
<b>390</b>	29.29	31.49	28.1	31.8	31.09
<b>39</b>	32.36	35.43	30.89	33.49	34.08
<b>3.9</b>	35.8	38.21	32.98	35.4	36.44

**Table 2. Reagent RVD with RVD Enhancer and Reagent SRVD amplify 3.9 viral copies /  $\mu$ L SARS-CoV-2 spiked into human saliva.** Direct PCR mediated by (A) Reagent RVD with RVD Enhancer (samples processed at 95°C) and (B) Reagent

SRVD (samples processed at 95°C) resulted in comparable Ct values when 4 SARS-CoV-2 gene regions were amplified. No Ct values were detected for Reagent RVD with RVD Enhancer at 3.9~39 copies /  $\mu\text{L}$  for the Orf1a gene region. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

### Reagent RVD-E (Dry Swab)

Copies / $\mu\text{L}$	N1	N2	E	NJ	Orf1a
<b>39,000</b>	22.48	25.23	24.71	30.52	27.02
<b>3,900</b>	24.83	30.05	27.67	32.28	29.96
<b>390</b>	28.78	33.26	30.63	35.73	32.05
<b>39</b>	33.09	35.45	34.46	38.83	34.74
<b>3.9</b>	35.62	37.46	36.88	40.53	37.88

**Table 3. Reagent RVD-E amplifies 3.9 viral copies /  $\mu\text{L}$  SARS-CoV-2 dried onto synthetic swabs.** Direct PCR mediated by Reagent RVD-E (samples processed at 95°C) resulted in Ct values for all 5 tested SARS-CoV-2 gene regions. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

### Key Conclusions:

- Tested PCR<sub>opsis</sub><sup>™</sup> direct PCR reagents amplify SARS-CoV-2 when diluted to 3.9 copies /  $\mu\text{L}$  in transport medium, saliva, or dried onto swabs for 4 of 5 tested gene regions.
- Observed Ct readings for samples in transport medium and processed with Reagent RVD with RVD Enhancer and Reagent RVD-RT are comparable (+/- 3) when processed at 95°C vs. 25°C, respectively.
- Observed Ct readings for saliva samples processed with Reagent RVD with RVD Enhancer and Reagent SRVD are comparable (+/- 3). However, Reagent SRVD facilitates amplification of the Orf1a gene region <39 copies /  $\mu\text{L}$ . Suggesting Reagent SRVD allows for greater access to this gene region compared to Reagent RVD with RVD Enhancer.
- Viral samples dried onto swabs and processed with Reagent RVD-E result in comparable (+/- 3) Ct readings compared to transport medium and saliva samples processed with Reagent RVD with RVD Enhancer, Reagent RVD-RT, or Reagent SRVD.
- The selection of gene target(s) affects the observed limit of detection.
- Based on previous observations (data not shown), the observed Ct values may slightly vary (+ / - 3 Ct) depending on the brand of transport medium. This is due to variations in the composition of transport mediums that may affect amplification.

- Although not demonstrated in this report, it may be possible to detect SARS-CoV-2 at levels less than 3.9 copies /  $\mu\text{L}$  if 40~45 cycles are performed. This is suggested by observed Cts in the mid-30s for samples diluted to 3.9 viral copies /  $\mu\text{L}$ .

## Methods:

### Materials:

- ATCC: 2019 Novel Coronavirus (VR-1986HK)
- Promega: 1-Step GoTaq® RT-qPCR Systems
- IDT: SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay
- BD™ Universal Viral Transport (UVT)
- Phosphate Buffered Saline (PBS)
- Puritan: Sterile nylon flocced tip swab
- Entopsis:
  - PCRopsis™ Reagent RVD with RVD Enhancer
  - PCRopsis™ Reagent RVD-RT (with PCRopsis™ Activator)
  - PCRopsis™ Reagent RVD-E
  - PCRopsis™ Reagent SRVD
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

### Prepare diluted viral samples in transport medium or saliva:

- Samples used with Reagent RVD with RVD Enhancer, Reagent RVD-RT and Reagent SRVD
  1. Perform 1 : 10 serial dilutions of SARS-CoV-2 in transport medium or saliva
  2. Mix thoroughly

### Prepare diluted viral samples in PBS:

- Samples used with Reagent RVD-E
  1. Perform 1 : 10 serial dilutions of SARS-CoV-2 in PBS
  2. Mix thoroughly
  3. Absorb ~50  $\mu\text{L}$  of diluted virus onto a nylon flocced swab by submerging the tip into the liquid
  4. Let the swab dry at room temperature for 2 hours

### Studies with PCRopsis™ Reagent RVD with RVD Enhancer, Reagent RVD-RT and Reagent SRVD:

1. Thoroughly mix 20  $\mu\text{L}$  of reagent with 20  $\mu\text{L}$  of diluted viral samples in a thin walled tube (0.2 mL) and cap tube
  - a. For Reagent RVD-RT: mix 50  $\mu\text{L}$  Activator with 950  $\mu\text{L}$  Reagent RVD-RT (mixture is referred to as Reagent RVD-RT)
2. Incubate the reagent mixture with viral sample for 10 minutes and mix thoroughly
  - a. At 25°C for Reagent RVD-RT
  - b. At 95°C for Reagent RVD with RVD Enhancer and Reagent SRVD

3. Add 5  $\mu\text{L}$  of processed sample to 15  $\mu\text{L}$  of RT-qPCR mix.
  - a. RT-qPCR mixture:
    - i. Promega GoTaq® qPCR Master Mix, 2X: 10  $\mu\text{L}$
    - ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4  $\mu\text{L}$
    - iii. IDT primer / probe: 1.5  $\mu\text{L}$
    - iv. Nuclease-Free Water: 3.1  $\mu\text{L}$
    - v. Processed sample: 5  $\mu\text{L}$
4. Run samples on qPCR Thermocycler for 45 cycles.
  - a. Reverse Transcription: 45°C for 15 minutes, then 95°C for 2 minutes
  - b. DNA Amplification: 95°C 30 seconds, 55°C 30 seconds for 45 cycles
  - c. Extension: 55°C 60 seconds
  - d. Hold: 4°C

#### Studies with PCR<sub>opsis</sub>™ Reagent RVD-E:

1. Add 100  $\mu\text{L}$  of Reagent RVD-E to a 1.5 mL tube
2. Place swab with dried viral sample into 1.5 mL tube
3. Vortex tube with swab for ~30 seconds, 3 times to elute sample
4. Transfer 50  $\mu\text{L}$  of eluted sample into a thin walled tube (0.2 mL) and cap tube
5. Incubate the reagent mixture with viral sample for 10 minutes at 95°C and mix thoroughly
6. Add 5  $\mu\text{L}$  of processed sample to 15  $\mu\text{L}$  of RT-qPCR mix.
  - a. RT-qPCR mixture:
    - i. Promega GoTaq® qPCR Master Mix, 2X: 10  $\mu\text{L}$
    - ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4  $\mu\text{L}$
    - iii. IDT primer / probe: 1.5  $\mu\text{L}$
    - iv. Nuclease-Free Water: 3.1  $\mu\text{L}$
    - v. Processed sample: 5  $\mu\text{L}$
7. Run samples on qPCR Thermocycler for 45 cycles.
  - a. Reverse Transcription: 45°C for 15 minutes, then 95°C for 2 minutes
  - b. DNA Amplification: 95°C 30 seconds, 55°C 30 seconds for 45 cycles
  - c. Extension: 55°C 60 seconds
  - d. Hold: 4°C

#### **References:**

1. Pan, Y. et. al. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infectious Diseases* (2020)
2. Wang, W. et. al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* (2020)
3. Zheng, S. et. al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ* (2020)
4. Hasanoglu, I. et. al. Higher viral loads in asymptomatic COVID-19 patients might be the invisible part of the iceberg. *Infection* (2020)