PCR*opsis*™ Oral Rinse Offers In-Transit Nucleic Acid Extraction of Bacteria and Viruses

Francis Buan Hong Lim[1,5], Abhignyan Nagesetti[1,6], Kevin Moreno[1,6], Agustin Galecio[1], Ian Cheong[1,2,3,4,5], Obdulio Piloto[1,5,6]

Affiliations
[1] Entopsis, Inc., USA
[2] Temasek Life Sciences Laboratory, Singapore
[3] Department of Biological Sciences, National University of Singapore, Singapore
[4] Pathnova Laboratories Pte Ltd, Singapore
[5] Protean Labs LLC, USA
[6] Polarity Ventures LLC, USA

Correspondence to: info@entopsis.com

Abstract:
Oral samples represent a non-invasive and convenient means of collecting material for PCR testing and other applications. In this report we highlight PCR*opsis*™ Oral Rinse, a collection and transport device for oral samples that mediates nucleic acid extraction while the oral sample is in transit from a collection site to a testing facility. This allows for the inclusion of such samples into PCR master mixes, without the time consuming and laborious process of nucleic acid extraction. PCR*opsis*™ Oral Rinse mediates complete / partial in-transit nucleic acid extraction for bacteria commonly found in oral samples and a RNA viruses. This capability further enhances the utility of the Oral Rinse, and positions it as the ideal collection and transport device for oral samples.

Results:

Saliva	Oral Rinse	Sample Processing	Sample added to PCR	Bacteria Ct Values
50%	50%	none	2.5 µL	14.05
50%	50%	none	5 µL	14.25
50%	50%	Reagent RVD with RVD Enhancer	5 µL	14.48
60%	40%	none	2.5 µL	14.16
60%	40%	none	5 µL	14.69
60%	40%	Reagent RVD with RVD Enhancer	5 µL	14.67
70%	30%	none	2.5 µL	14.76
70%	30%	none	5 µL	15.85
70%	30%	Reagent RVD with RVD Enhancer	5 µL	14.82

Figure 1. PCR*opsis*™ Oral Rinse performs in-transit DNA extraction of oral bacteria resulting in extraction-free PCR. PCR*opsis*™ Oral Rinse is mixed with saliva at indicated concentrations and stored at room temperature for ~36 hours. Samples were then used directly into the PCR master mix or processed with PCR*opsis*™ Reagent RVD with RVD Enhancer before being added to the PCR master mix. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Saliva	Carrier	Colonies per Plated Volume			Average % Inhibition
		1 uL	10 uL	100 uL	
50%	PBS	120	1200	12,000	N / A
50%	Oral Rinse	0	0	0	**100%**
60%	PBS	380	3800	38,000	N / A
60%	Oral Rinse	2	32	214	**99.36**
70%	PBS	530	5300	53,000	N / A
70%	Oral Rinse	14	164	2200	**96.71%**

Figure 2. PCR*opsis*™ Oral Rinse exhibits strong anti-microbial activity, even when diluted more than 2-fold. PCR*opsis*™ Oral Rinse or saline buffer (control), at 50%, 40%, and 30%, was mixed with 50%, 60% and 70% fresh human saliva and incubated at room temperature for 48 hours. Saliva samples diluted with PCR*opsis*™ Oral Rinse were then mixed with Luria Broth (LB) at indicated volumes up to 100 µL (e.g., 1 µL diluted saliva + 99 µL LB, 10 µL diluted saliva + 90 µL LB). 100 µL of samples were plated onto LB agar plates and cultured at 37˚C for 24 hours, at which point colonies were counted.

Saliva	Oral Rinse	Sample Processing	Sample added to PCR	SARS-CoV-2 (3900 copies / µL)	
				N1	N2
50%	50%	none	2.5 µL	26.46	28.24
50%	50%	none	5 µL	26.68	28.57
50%	50%	10 minutes at 95˚C	2.5 µL	24.52	27.32
50%	50%	10 minutes at 95˚C	5 µL	24.55	27.53
60%	40%	none	2.5 µL	28.55	30.36
60%	40%	none	5 µL	28.14	30.48
60%	40%	10 minutes at 95˚C	2.5 µL	24.61	27.25
60%	40%	10 minutes at 95˚C	5 µL	24.95	27.92
70%	30%	none	2.5 µL	30.38	32.69
70%	30%	none	5 µL	34.34	36.23
70%	30%	10 minutes at 95˚C	2.5 µL	24.83	27.96
70%	30%	10 minutes at 95˚C	5 µL	25.26	28.36

Figure 3. PCR*opsis*™ Oral Rinse performs in-transit RNA extraction of SARS-CoV-2 when heated at 95˚C for 10 minutes resulting in extraction-free PCR. PCR*opsis*™ Oral Rinse is mixed with saliva at indicated concentrations, spiked with SARS-CoV-2, and stored at room temperature for ~36 hours. Samples were then used directly into the PCR master mix or heated at 95˚C for 10 minutes before being added to the PCR master mix. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Saliva	Oral Rinse	Sample Processing	Sample added to PCR	SARS-CoV-2 (3900 copies / µL)	
				N1	N2
50%	50%	10 minutes at 95˚C	2.5 µL	24.52	27.32
50%	50%	10 minutes at 95˚C	5 µL	24.55	27.53
50%	50%	Reagent RVD with RVD Enhancer	5 µL	23.47	24.61
50%	50%	Reagent RVD-RT	5 µL	23.68	24.33
60%	40%	10 minutes at 95˚C	2.5 µL	24.61	27.25
60%	40%	10 minutes at 95˚C	5 µL	24.95	27.92
60%	40%	Reagent RVD with RVD Enhancer	5 µL	23.26	24.81
60%	40%	Reagent RVD-RT	5 µL	23.52	24.65
70%	30%	10 minutes at 95˚C	2.5 µL	24.83	27.96
70%	30%	10 minutes at 95˚C	5 µL	25.26	28.36
70%	30%	Reagent RVD with RVD Enhancer	5 µL	23.87	25.22
70%	30%	Reagent RVD-RT	5 µL	23.83	25.72

Figure 4. Lower Ct values may be observed with some gene sites when oral samples in PCR*opsis*™ Oral Rinse are processed with PCR*opsis*™ Reagent RVD with RVD Enhancer or PCR*opsis*™ Reagent RVD-RT. PCR*opsis*™ Oral Rinse is mixed with saliva at indicated concentrations, spiked with SARS-CoV-2, and stored at room temperature for ~36 hours. Samples were then heated at 95˚C for 10 minutes or processed with PCR*opsis*™ Reagent RVD with RVD Enhancer or PCR*opsis*™ Reagent RVD-RT before being added to the PCR master mix. Each sample was tested in duplicates and the indicated Ct is the average of two readings.

Key Conclusions:

- **Bacteria:** PCR*opsis*™ Oral Rinse mediates in-transit nucleic acid extraction of bacteria at room temperature; oral samples in PCR*opsis*™ Oral Rinse can be used directly into PCR master mixes without additional processing
    - Processing oral samples with PCR*opsis*™ Reagent RVD with RVD Enhancer did not result in lower Ct values, indicating most of the DNA was made available for amplification by PCR*opsis*™ Oral Rinse
    - Further studies are needed to determine whether the same result would be observed when amplifying a specific bacteria species, instead of using degenerate bacterial primers

- PCR*opsis*™ Oral Rinse inhibits the growth of 96%~100% of oral bacteria; depending on the ratio of saliva to Oral Rinse

- **RNA Virus:** PCR*opsis*™ Oral Rinse mediates in-transit nucleic acid extraction of RNA viruses at room temperature; oral samples in PCR*opsis*™ Oral Rinse can be used directly into PCR master mixes after heating at 95˚C for 10 minutes
    - Oral samples in PCR*opsis*™ Oral Rinse containing viruses can be used directly into PCR master mixes, but the sensitivity worsens as the ratio of saliva to Oral Rinse increases. Heating the oral sample in Oral Rinse results in significantly improved Ct values.
    - Processing oral samples in PCR*opsis*™ Oral Rinse with PCR*opsis*™ Reagent RVD with RVD Enhancer or PCR*opsis*™ Reagent RVD-RT results in improved amplification of target sequences, especially when the ratio of saliva to Oral Rinse is high (e.g., 70% saliva to 30% Oral Rinse).

- PCR*opsis*™ Oral Rinse mediates in-transit nucleic acid extraction, however the user should determine if additional sample processing is necessary for their application before adding the sample into their PCR master mix.

November 1, 2022 updates:

- A lack of amplification was observed with select saliva samples incubated with PCR*opsis*™ Oral Rinse, and processed with PCR*opsis*™ Reagent RVD-RT (data not shown). As such, we do not recommend using PCR*opsis*™ Reagent RVD-RT for oral samples in PCR*opsis*™ Oral Rinse.

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
    - Bacteria degenerate primers (R = A/G)
        - Forward: 5'- GGAGGCAGCAGT**RR**GGAAT -3'
        - Reverse: 5'- CTACC**R**GGGTATCTAATCC - 3'
- Entopsis Inc.:
    - PCR*opsis*™ Reagent RVD-RT (with PCR*opsis*™ Activator)
    - PCR*opsis*™ Reagent RVD with RVD Enhancer
    - PCR*opsis*™ Oral Rinse
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

Studies with bacteria:

1. Add oral sample to 15 µL of qPCR mix.
    a. qPCR mixture:
        i. 5x PCR Mix: 4 µL
        ii. 100x Lumiprobe SYBR Green: 0.2 µl
        iii. Degenerate primer - F: 1 µl
        iv. Degenerate primer - R: 1 µl
        v. Nuclease-Free Water: balance
2. Run samples on qPCR Thermocycler for 45 cycles.
    a. DNA Amplification: 95˚C for 10 minutes and the 95˚C 30 seconds, 55˚C 30 seconds, 72˚C 30 seconds for 45 cycles
    b. Extension: 72˚C 60 seconds
    c. Hold: 4˚C

Studies with PCR*opsis*™ Reagent RVD with RVD Enhancer:

3. Invert the bottle of Reagent RVD with RVD Enhancer to homogenize
4. Thoroughly mix 20 µL Reagent RVD with RVD Enhancer with 20 µL of oral samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes
5. Incubate Reagent RVD with RVD Enhancer mixture with oral sample for 10 minutes at 95˚C
6. Add heated sample to 15 µL of RT-qPCR mix.
    a. RT-qPCR mixture:
        i. Promega GoTaq® qPCR Master Mix, 2X: 10 µl
        ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4 µl
        iii. IDT primer / probe: 1.5 µl
        iv. Nuclease-Free Water: balance
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 5 seconds, 55˚C 15 seconds, 72˚C 15 seconds for 45 cycles
c. Extension: 72˚C 60 seconds
d. Hold: 4˚C

Studies with PCR*opsis*™ Reagent RVD-RT:

1. Thoroughly mix 950 µL Reagent RVD-RT with 50 µL Activator
    a. Referred to as just Reagent RVD-RT
2. Thoroughly mix 20 µL Reagent RVD-RT with 20 µL of oral samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes
3. Incubate Reagent RVD-RT mixture with oral sample for 10 minutes at 25˚C
4. Add oral sample to 15 µL of RT-qPCR mix.
    a. RT-qPCR mixture:
        i. Promega GoTaq® qPCR Master Mix, 2X: 10 µl
        ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4 µl
        iii. IDT primer / probe: 1.5 µl
        iv. Nuclease-Free Water: balance
5. 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 5 seconds, 55˚C 15 seconds, 72˚C 15 seconds for 45 cycles
    c. Extension: 72˚C 60 seconds
    d. Hold: 4˚C