PCR*opsis*™ Oral Rinse Offers Prolonged Product and Sample Stability While Exhibiting Anti-Microbial Properties and Mediating Direct PCR

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Abstract:
Oral samples provide a non-invasive and convenient means of testing people for a variety of medical conditions. In this report we highlight PCR*opsis*™ Oral Rinse, a device used to collect, transport, and store oral samples for PCR testing. This oral rinse retains its functionality when stored at room temperature for over 6 months. Oral samples collected with this product are stable at 4˚C for at least 2 months and at -20˚C for at least 5 months. PCR*opsis*™ Oral Rinse also inhibits ~100% of bacterial growth when mixed 1:1 with saliva, and inhibits ~96% bacterial growth in a 30% oral rinse – 70% saliva mixture when stored at room temperature for 48 hours. This microbial inhibition is observed without the test subject adding a secondary reagent to the sample post sample collection; thereby facilitating sample collection and improving consistency. Moreover, PCR*opsis*™ Oral Rinse is uniquely compatible with direct PCR approaches that help streamline testing, while reducing costs. As far as we know, there are no other oral sample collection and transport devices that are compatible with direct PCR.

Results:

		37°C		4°C		-20°C		Room Temperature	
		N1 (Duplicates)	N2 (Duplicates)	N1 (Duplicates)	N2 (Duplicates)	N1 (Duplicates)	N2 (Duplicates)	N1 (Duplicates)	N2 (Duplicates)
Day 0	**Reagent RVD w/ Enhancer**	22.41	22.59	22.41	22.59	22.41	22.59	22.41	22.59
	Reagent RVD-RT	23.42	23.72	23.42	23.72	23.42	23.72	23.42	23.72
Day 7	**Reagent RVD w/ Enhancer**	23.64	24.09	23.56	23.35	23.76	23.46	22.48	23.39
	Reagent RVD-RT	24.19	23.74	22.99	23.79	23.56	24.1	23.7	23.36
Day 14	**Reagent RVD w/ Enhancer**							22.89	23.86
	Reagent RVD-RT							23.27	23.44
Day 28	**Reagent RVD w/ Enhancer**	22.98	23.58	23.19	23.68	22.78	23.91	22.66	22.91
	Reagent RVD-RT	23.35	23.88	23.46	23.72	23.49	23.61	23.58	23.32
Day 56	**Reagent RVD w/ Enhancer**	23.53	24.15	22.34	23.71	23.71	24.65	22.93	23.77
	Reagent RVD-RT	23.27	23.95	24.37	24.78	23.3	24.63	22.98	23.56
Day 90	**Reagent RVD w/ Enhancer**	25.58	27.29	23.55	24.81	24.5	25.23	24.01	25.13
	Reagent RVD-RT	30.35	32.66	23.63	24.01	24.38	25.45	24.34	25.07
Day 112	**Reagent RVD w/ Enhancer**	25.9	26.91	22.48	23.87	22.36	23.16	22.92	23.57
	Reagent RVD-RT	28.54	30.29	24.52	25.15	23.34	24.48	23.78	24.43
Day 140	**Reagent RVD w/ Enhancer**	28.07	30.85	23.22	24.15	23.58	24.74	23.77	24.55
	Reagent RVD-RT	29.95	31.78	23.54	24.62	23.08	24.16	23.44	24.28
Day 168	**Reagent RVD w/ Enhancer**	30.41	33.24	23.37	24.79	23.39	24.07	23.74	24.06
	Reagent RVD-RT	31.23	35.61	23.66	24.82	23.15	24.48	23.52	24.87

Figure 1. PCR*opsis*™ Oral Rinse can be stored at room temperature, 4˚C, or -20˚C for at least 168 days and at 37˚C for at most 2 months without losing functionality. PCR*opsis*™ Oral Rinse is stored at indicated temperatures and then mixed 1:1 with fresh human saliva and SARS-CoV-2 (3,900 copies / µL) at indicated days. This mixture is then subjected to RT-qPCR amplifying regions of the N1 and N2 genes on SARS-CoV-2. Each sample was tested in duplicates and the indicated Ct is the average of two readings. Ct values in red indicate an increase of more than 2.5 Ct from baseline.

		Room Temperature		4°C		-20°C	
		N1 (Duplicates)	N2 (Duplicates)	N1 (Duplicates)	N2 (Duplicates)	N1 (Duplicates)	N2 (Duplicates)
Day 0	**Reagent RVD w/ Enhancer**	22.41	22.59	22.41	22.59	22.41	22.59
	Reagent RVD-RT	23.42	23.72	23.42	23.72	23.42	23.72
Day 7	**Reagent RVD w/ Enhancer**	22.78	23.76	22.6	23.6	23.4	23.49
	Reagent RVD-RT	24.7	26.35	23.67	23.52	23.69	24.06
Day 14	**Reagent RVD w/ Enhancer**	22.68	24.2	22.23	23.67	23.78	24.72
	Reagent RVD-RT	24.57	25.96	23.38	23.06	23.46	24.62
Day 21	**Reagent RVD w/ Enhancer**	24.31	26.03	23.35	24.06	23.5	24.59
	Reagent RVD-RT	29.67	31.36	24.1	24.55	23.44	24.06
Day 28	**Reagent RVD w/ Enhancer**	24.93	26.75	23.63	24.48	24.18	24.87
	Reagent RVD-RT	29.15	32.72	24.56	24.77	24.69	24.8
Day 35	**Reagent RVD w/ Enhancer**	26.42	29.43	23.42	24.6	23.97	25.03
	Reagent RVD-RT	30.43	33.08	24.32	24.49	23.85	24.74
Day 42	**Reagent RVD w/ Enhancer**	26.65	29.42	23.56	23.72	24.08	24.89
	Reagent RVD-RT	30.02	32.98	24.1	24.35	24.23	24.59
Day 49	**Reagent RVD w/ Enhancer**	26.88	29.48	23.33	24.7	24.31	25.8
	Reagent RVD-RT	32.32	35.33	24.64	24.43	24.58	25.48
Day 56	**Reagent RVD w/ Enhancer**	27.53	30.15	23.61	24.1	23.79	25.35
	Reagent RVD-RT	33.73	None	24.89	24.75	23.39	25.01
Day 63	**Reagent RVD w/ Enhancer**	27.45	31.87	23.11	23.43	23.11	24.88
	Reagent RVD-RT	33.47	None	24.17	23.75	23.29	25.32
Day 70	**Reagent RVD w/ Enhancer**	27.65	31.41	23.28	23.82	23.28	25.08
	Reagent RVD-RT	None	None	24.51	24.23	23.54	24.79

Figure 2. PCR*opsis*™ Oral Rinse + saliva + SARS-CoV-2 can be stored at room temperature for less than seven days but at 4˚C or -20˚C for at least 70 days. PCR*opsis*™ Oral Rinse mixed 1:1 with fresh human saliva and SARS-CoV-2 (3,900 copies / µL) is stored at indicated temperatures for indicated days. This mixture is then subjected to RT-qPCR amplifying regions of the N1 and N2 genes on SARS-CoV-2. Each sample was tested in duplicates and the indicated Ct is the average of two readings. Ct values in red indicate an increase of more than 2.5 Ct from baseline.

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 3. 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.

Key Conclusions:

- PCR*opsis*™ Oral Rinse can be stored at room temperature, 4˚C, or -20˚C for at least 6 months without losing functionality
- PCR*opsis*™ Oral Rinse can withstand storage at high temperatures, 37˚C, for at most ~60 days before the functionality of the product is compromised
- Oral samples collected with PCR*opsis*™ Oral Rinse can remain at room temperature for at most 7 days when samples are going to be processed with PCR*opsis*™ Reagent RVD-RT
- Oral samples collected with PCR*opsis*™ Oral Rinse can remain at room temperature for at most 14 days when samples are going to be tested with PCR*opsis*™ Reagent RVD with RVD Enhancer
- Oral samples collected with PCR*opsis*™ Oral Rinse can be stored for at least 70 days at 4˚C or -20˚C without compromising results when using PCR*opsis*™ Reagent RVD with RVD Enhancer or PCR*opsis*™ Reagent RVD-RT for direct PCR
- PCR*opsis*™ Oral Rinse has anti-microbial activity against bacteria commonly found in the human mouth, without the addition of a secondary reagent post sample collection

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.

- Recommendations for oral samples collected with PCR*opsis*™ Oral Rinse:
  - should be stored chilled (4˚C ~ 10˚C) after collection for consistency; especially if the concentration of saliva is >50% of the total volume
  - should be transported chilled (4˚C ~ 10˚C) to testing facility for consistency; especially if the concentration of saliva is >50% of the total volume
  - samples can be stored for a prolonged period of time at 4˚C or -20˚C; -20˚C is expected to be preferred when storing samples for over 3 months

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

1. Invert the bottle of Reagent RVD with RVD Enhancer to homogenize
2. 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
3. Incubate Reagent RVD with RVD Enhancer mixture with oral sample for 10 minutes at 95˚C
4. Add 5 µL of the 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: 3.1 µl

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

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 5 µL of Reagent RVD-RT with 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: 3.1 µl
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