PCR*opsis*™ Reagent RVD-E Enables Extraction-free Amplification of Gene Targets from Specimens on Swabs

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Abstract:
Traditional collection, transport, and testing of clinical samples require numerous consumables; this presents supply chain and cost bottlenecks. Here we present an alternative approach that eliminates the use of transport medium and nucleic acid extraction kits. Clinical samples from a body site (e.g., nasopharyngeal, oral, skin, vaginal, etc.) are collected on sterile, synthetic swabs and shipped in sterile, empty transport tube to a testing location. PCR*opsis*™ Reagent RVD-E is then added to the transport tube containing the swab and vortexed to elute the associated clinical material directly from the swab. The eluted sample is heated to release RNA / DNA and ensure they are accessible for down stream applications (e.g., qPCR, RT-qPCR, etc.). This approach was successfully used to amplify residual human cells, SARS-CoV-2 (at 3.9 genome copies / µL), Staphylococcus aureus (at 92 cfu / mL) and Pseudomonas aeruginosa (at 51 cfu / mL). PCR*opsis*™ Reagent RVD-E offers a simple, cost effective means of collecting, transporting and testing diverse specimens.

Results:

A) Titered SARS-CoV-2

Copies / µL	N1	N2	E	NJ	Orf1a
39,000	22.48	25.23	24.71	30.52	27.02
3,900	24.83	30.05	27.67	32.28	29.96
390	28.78	33.26	30.63	35.73	32.05
39	33.09	35.45	34.46	38.83	34.74
3.9	35.62	37.46	36.88	40.53	37.88

B) COVID-19 clinical samples

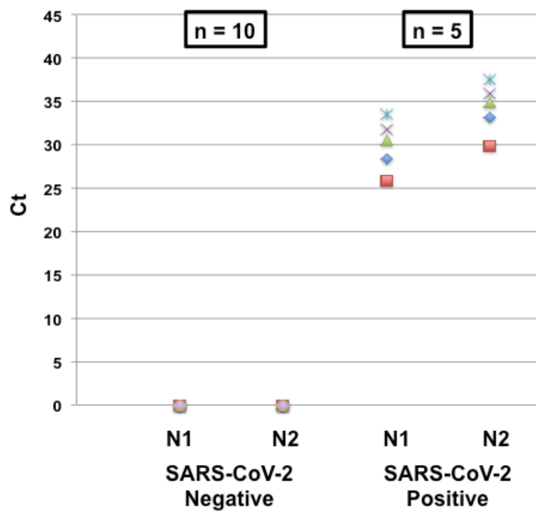
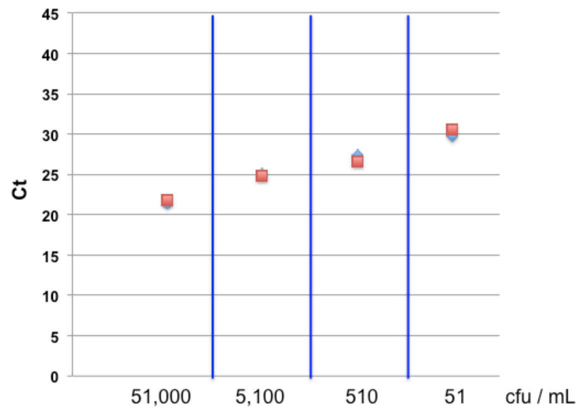


Figure 1. Reagent RVD-E facilitates viral RNA extraction-free amplification of 5 gene sites from heat inactivated SARS-CoV-2 and clinical samples dried onto nylon swabs. A) All gene sites were amplified from eluted swab specimens containing 39,000 to 3.9 genome copies / mL of titered SARS-CoV-2. Note: the stated viral genome concentration assumes 100% of bound virus is eluted from the swab, which is not practical and the eluted sample likely contains fewer genomes than what is stated. Study was performed in duplicates and averages shown. B) All 15 tested clinical samples (10 negative and 5 positive for SARS-CoV-2) were correctly identified using Reagent RVD-E + RT-qPCR.

A) Serial dilution: P. auerginosa



B) Replicates at LoD: P. auerginosa

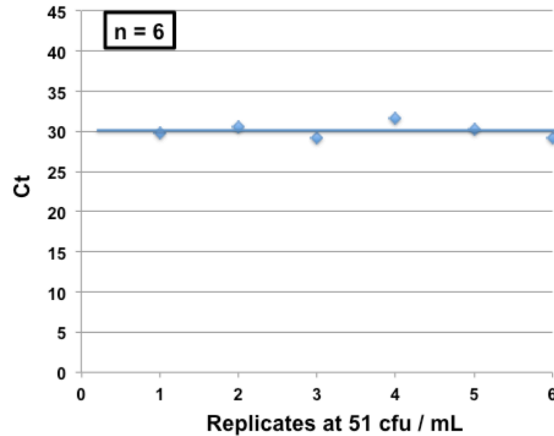
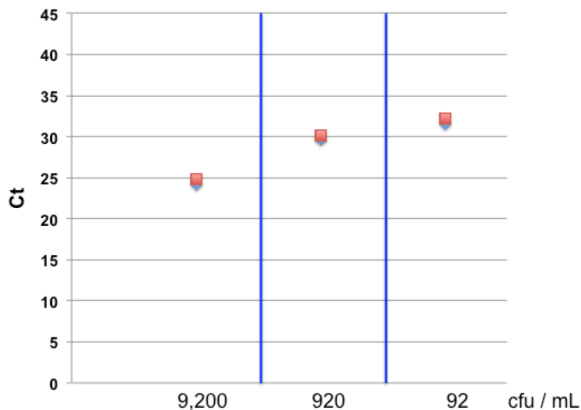


Figure 2. Reagent RVD-E facilitates DNA extraction-free amplification of P. auerginosa dried onto nylon swabs with a LoD <100 cfu / mL. (A) Amplification of bacteria specific gene fragment was reliably observed at microbial concentrations of 51,000 – 51 cfu / mL with Ct values between 22-31 when duplicate samples were tested. (B) All 6 replicate samples at 51 cfu / mL resulted in amplification.

A) Serial dilution: S. aureus



B) Replicates at LoD: S. aureus

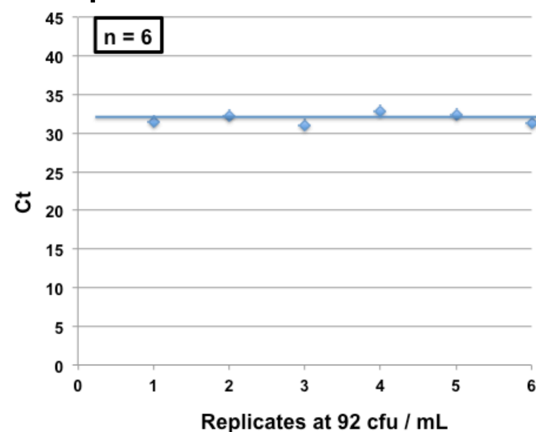


Figure 3. Reagent RVD-E facilitates DNA extraction-free amplification of S. aureus dried onto nylon swabs with a LoD <100 cfu / mL. (A) Amplification of bacteria specific gene fragment was reliably observed at microbial concentrations of 9,200 – 92 cfu / mL with Ct values between 24-33 when duplicate samples were tested. (B) All 6 replicate samples at 92 cfu / mL resulted in amplification.

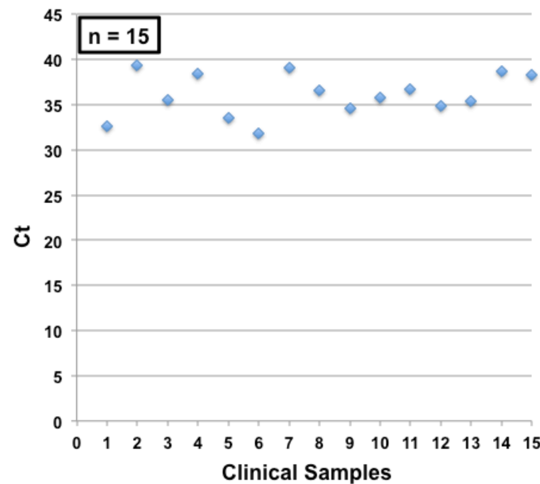


Figure 4. Residual human cells from nasopharyngeal samples dried onto nylon flocked swabs can be detected using Reagent RVD-E + RT-qPCR. Human RNase P was detected in all 15 tested clinical samples dried onto nylon flocked swabs with Cts 30~40.

Conclusions & Discussion:

- Reagent RVD-E + RT-qPCR resulted in amplification of all tested gene sites in all samples containing SARS-CoV-2 with Cts <40 (Figure 1A & 1B). We observed viral titer dependent amplification of relevant genes.

- Reagent RVD-E + RT-qPCR amplified swab samples with 3,900 viral copies / mL dried onto the swab (Figure 1A). FDA emergency use authorization (EUA) approved direct swab tests for SARS-CoV-2 report a limit of detection between 60,000 ~ 540,000 NDU / mL[1]. As such, Reagent RVD-E + RT-qPCR has a lower limit of detection compared to commercial tests.

- It should be noted that the reported concentration of SARS-CoV-2 corresponds to the concentration of virus in the original test sample and assumes 100% elution efficiency from the swab. This elution efficiency may not be practically possible. As such, the concentration of viral genome copies in the eluted sample is likely lower than the stated 3.9 genome copies / µL. Therefore, less than 3.9 genome copies / µL may be detectable.

- SARS-CoV-2 was dried onto nylon flocked swab samples for ~48 hours to mimic the time between sample collection, transport and delayed testing. In comparison, the FDA recommends a minimum period of 20 minutes for samples to dry onto test swabs[1]. The longer drying period used in this study may result in viral RNA degradation and loss of detectable gene targets. As such, the presented data aims to mimic real-world conditions instead of optimized laboratory conditions that produce artificially low sensitivities.

- Reagent RVD-E + qPCR was able to amplify species-specific gene targets from both gram positive and negative bacteria, respectively S. aureus and P. aeruginosa, bound to nylon flocked swabs (Figure 2A & 3A). The observed limit of detection for both bacteria was **<100 cfu / mL** in the eluted sample assuming 100% elution efficiency from the swab (Figure 2B & 3B).

- Reagent RVD-E + RT-qPCR was successful in amplifying human RNase P from 100% of tested clinical samples (Figure 4). As such, this data confirms the ability of the product to amplify residual human gene targets.

- The data supports the use of Reagent RVD-E for the amplification of viral, bacterial and mammalian gene targets directly from synthetic swabs, without the need for transport mediums. More extensive studies are needed to confirm the utility of Reagent RVD-E in the clinical setting.

Methods:

Materials:

- Microorganisms (ATCC): Heat inactivated 2019 Novel Coronavirus (ATCC: VR-1986HK,2019-nCoV/USA-WA1/2020), Staphylococcus aureus, Pseudomonas aeruginosa
- Heat inactivated remnant clinical samples in universal transport medium
- Nylon flocked swab
- Promega: 1-Step GoTaq® RT-qPCR Systems
- IDT: SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay
- IDT: DNA primers & probes (see Table 1)
- BD™ Universal Viral Transport (UVT) medium
- Entopsis:
    - PCR*opsis*™ Reagent RVD-E
    - PCR*opsis*™ 5x PCR Master Mix
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

Studies with Samples Dried on Swabs:

1. Prepare swabs with dried sample:
    a. Dilute test sample (e.g., virus, bacteria, clinical, etc.) in 50 µL of 10 mM Tris pH 8 at the desired concentration in a sterile tube
        i. Clinical samples previously confirmed positive or negative for SARS-CoV-2 were diluted 1:10
    b. Submerge a sterile nylon flocked swab into the test sample to absorb most / all of the liquid
    c. Let the sample-containing swabs dry at room temperature for 48 hours in a biosafety cabinet to mimic the delay between sample collection and testing
2. Elute material from swabs:
    a. Add 100 µL of PCR*opsis*™ Reagent RVD-E to a 10 mL transport tube

b. Submerge a dried, sample-containing swab into Reagent RVD-E
c. Break the swab so that the transport tube can be closed
d. Vortex for ~30 seconds, 3 times

3. Specimen lysis & nucleic acid stabilization:
    a. Transfer ~50 µL of eluted sample into a thin-walled PCR tube
    b. Heat for 10 minutes at 95˚C
    c. Let cool at room temperature for ~10 seconds before continuing
4. RT-qPCR / qPCR:
    a. Add 5 µL of heated Reagent RVD-E sample to 15 µL of RT-qPCR / qPCR mix
        i. RT-qPCR mixture:
            1. GoTaq® qPCR Master Mix, 2X: 10 µl
            2. 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4 µl
            3. IDT primer / probe: 1.5 µl
            4. Nuclease-Free Water: 3.1 µl
            5. Reagent RVD-E sample: 5 µL
        ii. qPCR mixture:
            1. PCR*opsis*™ 5x PCR Master Mix: 4 µL
            2. Forward primer: 1 µL
            3. Reverse primer: 1 µL
            4. Probe: 0.5 µL
            5. Nuclease-Free Water: 8.5 µl
            6. Reagent RVD-E sample: 5 µL
    b. Run samples on qPCR Thermocycler for 45 cycles
        i. Reverse Transcription: 45˚C for 15 minutes, then 95˚C for 2 minutes
        ii. DNA Amplification: 95˚C 30 seconds, 55˚C 30 seconds, 72˚C 30 seconds for 45 cycles
        iii. Extension: 72˚C 60 seconds
        iv. Hold: 4˚C

Table 1.

Microorganism	Primer	Probe	Sequence (5′-3′)
Staphylococcus aureus	S868F		CCACATGCCTCTAATAATG
	S1064R		GCGATTTTATTTTCTTTTGTAC
		S1024P	ATGCCATGCCTCCAAATATCGC
Pseudomonas aeurginosa	Pa23F		TCCAAGTTTAAGGTGGTAGGCTG
	Pa23R		CTTTTCTTGGAAGCATGGCATC
		Pa23P	AGGTAAATCCGGGGTTTCAAGGCC

References

1. SARS-CoV-2 Reference Panel Comparative Data (2020). Table 2B-Direct Swabs (Dry Swabs) https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative-data#table2