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Extraction-Free Testing for SARS-CoV-2 in Nasal Swab and Saliva Samples on a Single High-Throughput Platform

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Abstract

The COVID-19 pandemic introduced an urgent need for rapid and high-throughput testing for SARS-CoV-2. RNA extraction is a major bottleneck for RT-qPCR. We describe a semi-automated, extraction-free RT-qPCR assay for detection of SARS-CoV-2 in nasal swab and saliva samples on a single platform. With a limit of detection of 4 copies/mL, this laboratory developed test performed equivalently to established methods requiring nucleic acid extraction. Five technologists staffing two shifts per day (80 person-hours) processed more than 400,000 samples over 10 months. Patients opted to provide nasal swab samples (83.6%) more frequently than saliva (16.4%), creating the added challenge of producing swab collection kits. Real-world testing data indicated a higher frequency of SARS-CoV-2 detection in saliva (10.1%) compared to nasal swab (7.7%). This cost-effective and quickly scalable approach is suitable for pandemic preparedness planning related to surveillance and diagnostic testing.

Keywords: COVID-19; RNA; SARS-CoV-2; Saliva samples; Nasal swab

Introduction

The rapid global spread of SARS-CoV-2 underscored the need for high-throughput pathogen detection during future pandemics [1-3]. Due to the high rate of transmission from asymptomatic infected individuals, disease surveillance contributed significant sample volume to diagnostic testing [4-6]. As the primary pillar for infectious disease detection, RT-qPCR assays were designed worldwide to test a variety of sample types for an array of viral gene targets [7-10]. Current RT-qPCR methods are hampered by nucleic acid processing, including isolation and purification of total RNA, elution and concentration, and reverse-transcription of purified RNA to complementary DNA. Extraction is manually laborious and expensive and increases the risk of contamination and human error [2]. In periods of high demand, shortage of nucleic acid extraction supplies exacerbates the limitations of such viral detection methods [11,12]. We developed an assay for rapid, high-throughput, extraction-free RT-qPCR method for SARS-CoV-2 detection. This assay consists of 1) a standardized sample collection tube compatible with high-throughput processing of swab and saliva samples on a single platform, 2) development of an innovative buffer for stable nasal swab sample transport and processing, and 3) testing of the primary sample without extraction. These features overcome shortcomings of conventional RT-qPCR detection methods and can contribute significantly to pandemic preparedness planning.

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Materials and Methods

Collection devices

Self-collection of nasal swab and saliva samples was performed with instruction from and supervision by a trained healthcare worker. For nasal swab, each anterior naris was sampled using 10 rotations of a flocked nasopharyngeal or oropharyngeal swab with a 30 mm distal breakpoint, which was placed into a 2-mL cryogenic vial (NEST) pre-filled with 1 ml of viral transport buffer (VTB) consisting of tris-borate-EDTA (TBE) unless indicated otherwise for validation experiments. Saliva samples were collected into the same empty cryovials without VTB using a saliva funnel or collection aid (Salimetrics). Commercial collection devices (DNA Genotek) for swab (OR100) and saliva (OM505) were used as indicated.

Human samples

Human samples for test development and validation were collected according to Institutional Review Board approval (Salus #Summit-COVID-SLV-1) and provided by students, faculty, and staff at the University of Colorado as well as community members presenting to COVIDCheck Colorado testing sites throughout the state. Saliva samples used for clinical validation (20 positive and 20 negative) were remnant samples provided by a CLIA laboratory authorized to perform SalivaDirect™ and tested in a blind fashion. Clinical samples (n = 409,883) were also collected at COVIDCheck Colorado sites according to pre-analytical protocols requiring transport to the laboratory at ambient temperature within 24 hours.

Sample processing

Samples for validation and real-world clinical samples were collected into barcoded, externally threaded 2-mL cryovials (NEST) compatible with semi-automatic decapping/recapping instruments (Brooks FluidX Aperio or IntelliXcap). Following accessioning by barcode and placement into customized, 3D-printed 48-well racks, samples were rocked (60 rpm) at room temperature until transfer to a BSL2+ room. Two 48-well racks were decapped, and samples were transferred into 96-well plates using an electronic pipetting machine (Integra ViaFlow). For each nasal swab sample, 35 µL of VTB was mixed with 5 µL of proteinase K (PK, Promega). For each saliva swab sample, 30 µL of saliva was mixed with 5 µL of PK and 5 µL tris 2-carboxyethyl phosphine (TCEP) or another buffer as indicated for validation experiments. Sample plates were placed on a digital microplate shaker (500 rpm) for one minute and then into a thermal cycler at 95°C for five minutes for heat-inactivation [22]. Sample plates were tested immediately or held at 4°C.

SARS-CoV-2 RT-qPCR

Test validation was performed by comparing the extraction-free method to the CDC 2019-nCoV RT-qPCR assay [17] – both performed in the Summit Biolabs Liquid Biopsy Laboratory (CLIA ID 06D2213521; Aurora, CO) – or to SalivaDirect™ performed in a separate CLIA laboratory authorized by the Yale School of Public Health [10]. The extraction-free assay utilized primers and probes for the nucleocapsid (N1) viral gene and ribonuclease P (RNP) human gene in a single reaction as described by the CDC 2019-nCoV RT-qPCR assay [17]. N2 reagents were not used. 96-well testing plates were prepared by loading each well with 12.5 µL of RT-qPCR master mix, including 10 µL of Luna Universal Probe One-Step Reaction Mix (NEB), 1 µL of Luna Warm start RT enzyme Mix (NEB), and 1.5 µL of N1/RNP primers/probes (6.7 µM for primers, 1.7 µM FAM-labeled N1 probe, and 1.7 µM ATTO-647-labeled RNP probe, IDT). A 7.5 µL sample aliquot from each of 96 wells on the sample plate was then transferred to the corresponding well on the testing plate. The same volume was used for the positive control (IDT synthetic 2019-SARS-CoV-N control, 4000 copies/µL), negative control (IDT Hs-RPP30 control, 4000 copies/µL), and no-template control (water) on each testing plate. The 96-well testing plate was then analyzed on a CFX Opus 96 system (Bio-Rad) using the following thermal profile: Step 1: 55°C 10 minutes, 1 cycle; Step 2: 95°C 1 minute, 1 cycle; and Step 3: 95°C 10 seconds, 60°C 30 seconds, 40 cycles. Plates were assessed for signal in the FAM channel for the N1 target and Cy5 channel for the RNP target. Interpretation of Cq values for N1 and RNP targets was based on a Cq value threshold of 36 for N1 and 35 for RNP. SARS-CoV-2 was reported as detected for N1/FAM Cq ≤36, not detected for N1/FAM Cq >36 and RNP/Cy5 ≤35, or invalid for N1/FAM Cq >36 and RNP/Cy5 >35.

Results

Collection Device and Sample Preparation

Collection devices for swab and saliva samples were standardized for integration onto the same testing platform. Barcoded 2-mL cryogenic vials with an externally threaded screw cap were selected due to compatibility with semi-automatic decapping/recapping machines. For swab collection kits, cryovials were decapped, filled with viral transport buffer (VTB), and recapped for distribution (Figure 1A). At the site of patient collection, anterior nares samples were collected using a flocked nasopharyngeal or oropharyngeal swab with a proximal breakpoint 30 mm from the swab tip, enabling full immersion of the swab tip into VTB without impeding closure of the cryovial cap. For saliva samples, a funnel or collection aid was used for saliva collection directly into an empty cryovial (Figure 1B). Upon receipt in the CLIA laboratory, barcoded samples were accessioned, transferred to a BSL2+ room, arranged into 96-well format using a decapper and an electronic pipetting machine or multi-channel electronic equalizer, and then heat-inactivated (Figure 1C). In another BSL2+ biosafety cabinet, reaction plates were loaded with

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RT-qPCR master mix. Sample and reaction plates were then moved to the molecular microbiology laboratory where non-infectious patient samples were transferred to reaction plates using an electronic pipetting machine or multi-channel electronic equalizer (Figure 1D). Thus, standard collection devices containing swab or saliva samples were processed using a single laboratory workflow.

Buffer Optimization

To perform extraction-free RT-qPCR, a buffer that stabilizes nucleic acids and does not interfere with PCR is essential. Nucleic acid stability is improved by treatment with proteinase K (PK), which degrades proteins, including nucleases, in the sample [13-15]. A variety of buffer components and commercial collection devices were evaluated. Swab samples from healthy volunteers were collected into each buffer type, spiked with heat-inactivated SARS-CoV-2 virus, treated with PK, and tested by RT-qPCR. The SARS-CoV-2 N1 target was not detected in swabs immersed in EDTA or a commercial collection device (Figure 2A). Among swab matrices in which SARS-CoV-2 was detected, the tris-borate-EDTA (TBE) buffer produced the strongest signal (i.e. lowest cycle quantification (Cq) value) and was selected as the primary component of VTB for swab collection devices. Although saliva was collected into empty cryovials, buffer evaluation was performed to design a post-collection additive containing PK in the saliva testing protocol. SARS-CoV-2 was detected in saliva with all added buffers except for a commercial saliva collection device. Tris-2-carboxyethyl phosphine (TCEP) resulted in the lowest Cq in saliva samples (Figure 2B). Therefore, to optimize SARS-CoV-2 detection, nasal swabs were collected into cryovials pre-filled with a TBE-based VTB, and raw saliva samples were collected into empty cryovials and later treated with a TCEP-based solution during specimen processing.

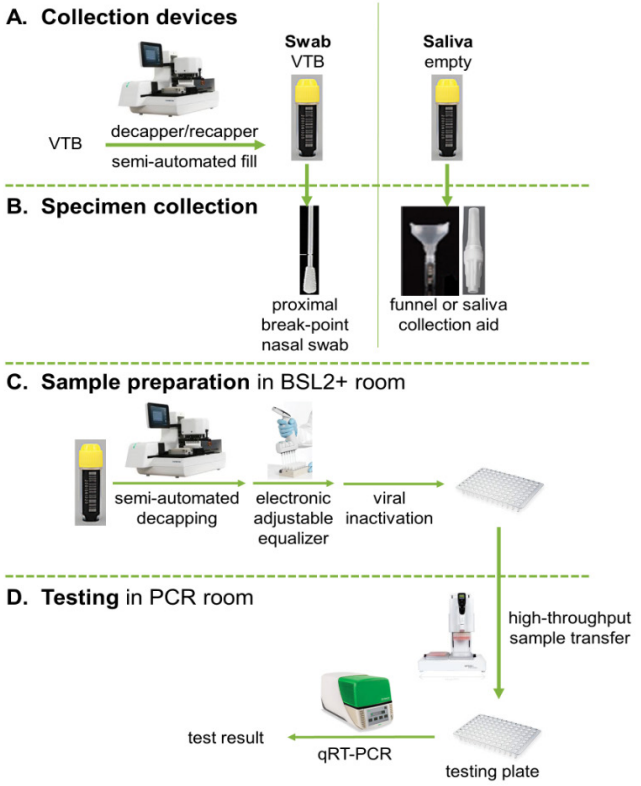

Figure 1: Rapid and high throughput testing of swab and saliva samples. (A) 2-mL cryovials with externally threaded caps were used for nasal swab and saliva collection kits. Cryovials for nasal swabs were semi-automatically decapped, loaded with viral transfer buffer (VTB), and recapped. Empty cryovials were used for saliva. **(B)** Cryovials pre-filled with VTB were paired with a proximal breakpoint swab to complete the nasal swab collection device. The saliva collection device included a funnel or saliva collection aid placed on top of an empty cryovial. **(C)** Sample preparation in the BSL2+ room was performed by decapping, aliquoting into 96-well plates using an Electronic Adjustable Tip Spacing Multichannel *Equalizer*, and recapping samples followed by heat inactivation of the sample plate. **(D)** Testing plates were prepared by loading with PCR master mix and samples were then transferred from the sample plate to testing plate for RT-qPCR testing.

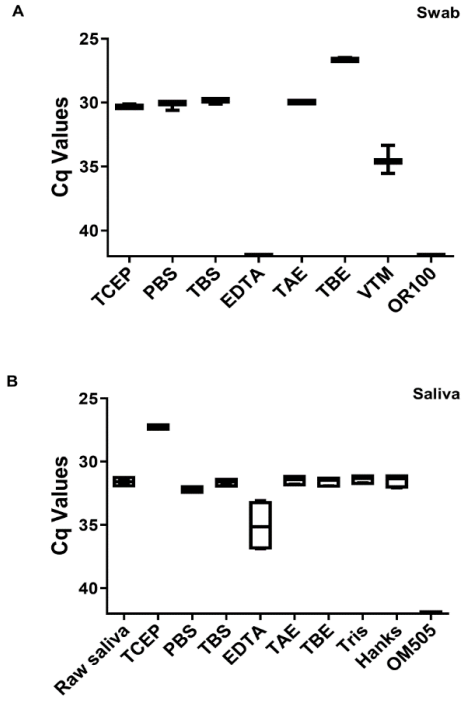

Figure 2: Optimizing buffer components for swab and saliva samples. Different buffer components were mixed with contrived samples containing 400 copies/mL of heat-inactivated SARS-CoV-2 virus for **(A)** nasal swab and **(B)** saliva. Cq values represent signal of the N1 primer/probe. OR100 is a commercial nasal swab collection device. OM505 is a commercial saliva collection device. TCEP, tris(2-carboxyethyl)phosphine; PBS, phosphate buffered saline; TBS, tris-buffered saline; EDTA, ethylenediaminetetraacetic acid; TAE, tris-acetate-EDTA; TBE, tris-borate-EDTA; VTM, commercial viral transport media; Hanks, Hanks' balanced salt solution.

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Stability of Viral Transport Buffer (VTB)

Since the swab collection tubes were pre-filled with VTB, the stability of VTB was assessed to determine the shelf-life of the collection device and the susceptibility of VTB to various conditions during transport. VTB stored at 4°C, room temperature, or 37°C was compared to fresh VTB following addition of a healthy swab sample and SARS-CoV-2 (4 - 4,000 copies/µL). The Cq value for each viral load was unaffected by VTB stored at these temperatures for at least 32 weeks (Figure 3A). Similarly, VTB stored at -80°C, -20°C and 4°C tested up to 3 weeks later did not impact Cq values (Figure 3B). Therefore, VTB demonstrated long-term stability at varying temperatures without impacting analytical sensitivity for SARS-CoV-2 RNA.

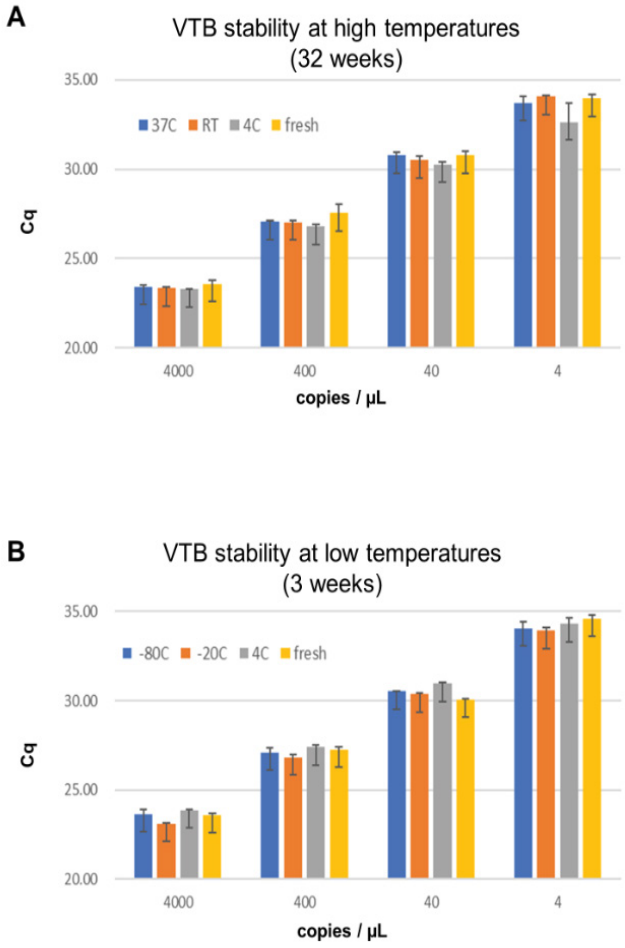

Figure 3: Stability of viral transport buffer (VTB) for nasal swab. (A) VTB was stored at 37°C (blue), room temperature (orange), or 4°C (gray) for up to 32 weeks or freshly made (yellow). Swab tips from healthy volunteers were placed into VTB, to which heat-inactivated SARS-CoV-2 was added at the indicated viral loads. Cq values represent signal of the N1 primer/probe. RT, room temperature. **(B)** As above for VTB stored at -80°C (blue), -20°C (orange), or 4°C (gray) for up to 3 weeks or freshly made (yellow).

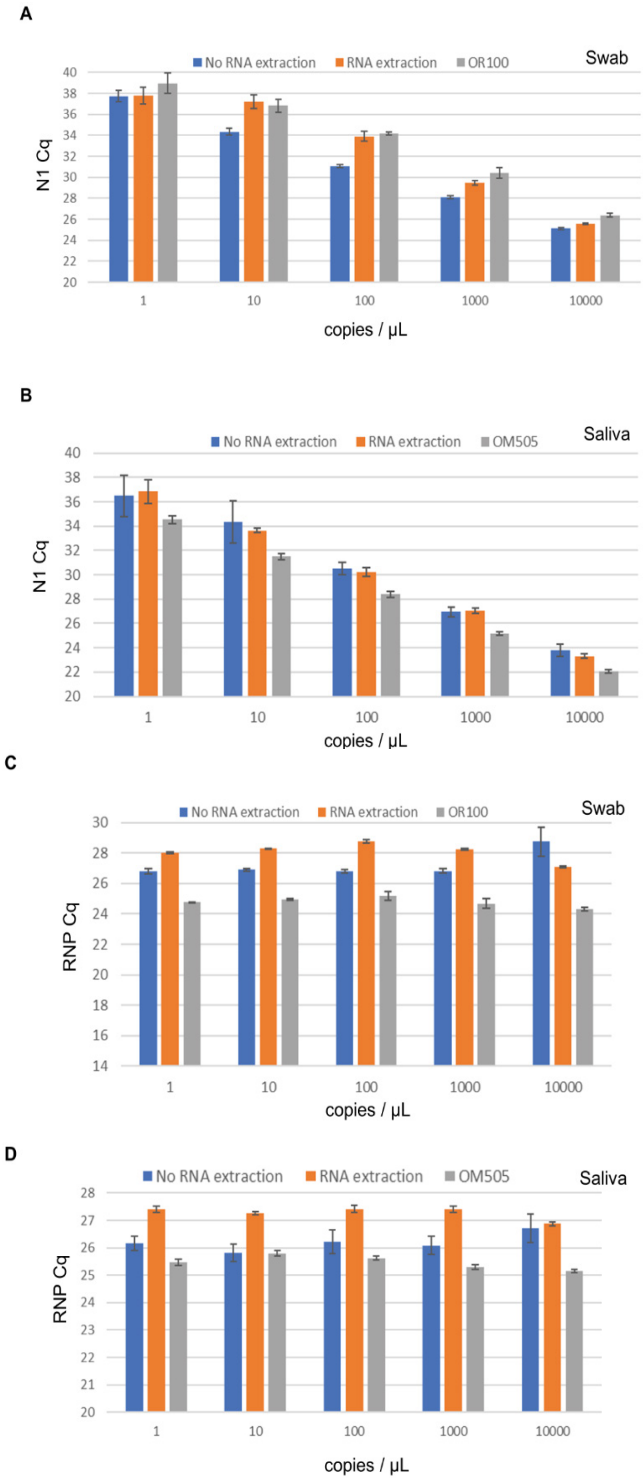

Figure 4: Comparison of RT-qPCR performance with and without RNA extraction. Nasal swab and saliva samples from healthy volunteers were collected into cryovials or commercial collection devices (OR100, OM505), which were spiked with heat-inactivated SARS-CoV-2 at the indicated viral loads. Cryovial samples were tested with extraction (orange) or without extraction (blue) in parallel to extracts from commercial collection devices (gray). **(A)** N1 Cq values of nasal swab, **(B)** N1 Cq values of saliva, **(C)** RNP Cq values of nasal swab, **(D)** RNP Cq values of saliva.

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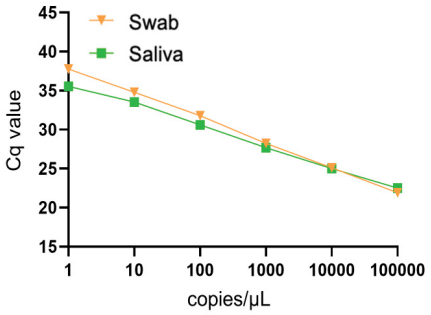
Analytical Validation

To perform analytical validation of the extraction-free RT-qPCR, swabs and saliva from healthy volunteers were collected in a cryovial with VTB as well as a commercial collection device. Saliva samples were collected into empty cryovials and a commercial collection device. Samples were then spiked with SARS-CoV-2 at a range of viral loads (1-10,000 copies/µL). Cryovial samples were either tested directly or after RNA extraction and samples in commercial collection devices were tested after RNA extraction. Extraction-free testing of swab and saliva samples demonstrated equivalent Cq values of the nucleocapsid N1 target compared to extracts from an identical cryovial or a commercial kit (Figure 4 A-B). Cq values of the quality control RNP target were not negatively impacted (Figure 4 C-D).

Limit of detection studies were performed by comparing samples serially diluted with heat-inactivated SARS-CoV-2 (1 - 100,000 copies/µL). The relationship between viral load and Cq value was linear on a log-log scale and equivalent between swab and saliva (Figure 5A). Testing of samples with viral loads of 0, 2, 4, 6, 8, 10, 50, and 100 copies/µL suggested a preliminary limit of detection at 4 copies/µL, with SARS-CoV-2 detection in 5 out of 6 samples using a N1 Cq threshold of 36. The limit of detection was confirmed by testing 20 additional replicates, overall demonstrating detection of 4 copies/µL in more than 95% of samples (Figure 5B).

A



B

	Viral Load (copies/µL)							
	100	50	10	8	6	4	2	0
Swab	6/6	6/6	6/6	6/6	6/6	25/26	3/6	0/6
Saliva	6/6	6/6	6/6	6/6	6/6	25/26	5/6	0/6

Figure 5: Limit of detection. (A) Testing of nasal swab (orange) and saliva (green) spiked with heat-inactivated SARS-CoV-2 at 100,000 copies/µL and serially diluted to 2 copies/µL. Cq values represent signal of the N1 primer/probe. **(B)** Results from testing six replicates of low viral load samples (# detected / # tested). An additional 20 replicates at 4 copies/µL were tested.

Clinical Validation

To confirm that assay performance was also robust from patients with COVID-19, 38 positive and 31 negative swab samples were tested directly by the extraction-free protocol or extracted and tested according to the Centers for Disease Control and Prevention (CDC) protocol [16,17]. The positive percent agreement (PPA) and the negative percent agreement (NPA) were both 100% (Figure 6A). Similarly, 82 positive and 171 negative saliva samples demonstrated a PPA of 98.8% and NPA of 99.4% (Figure 6B). The extraction-free assay was also compared to the SalivaDirect™ protocol. Swabs collected from 185 consecutive patients presenting for diagnostic testing revealed a PPA of 83.3% and NPA of 99.4% compared to SalivaDirect™, although this comparison was limited by the small number of SARS-CoV-2 samples (Figure 7A). Extraction-free and SalivaDirect™ testing of 20 positive and 20 negative saliva samples demonstrated a PPA and NPA of 95.0% and 100.0%, respectively (Figure 7B).

Real-world Clinical Testing

This laboratory developed test was used to evaluate 409,883 samples from March through December of 2021. On average five technologists staffing two shifts per day (i.e. 80 person-hours daily) tested 1,350 samples per day. On days with high sample volumes, up to 7,000 tests were completed within 24 hours. Our laboratory prepared collection kits for swab and saliva, including pre-filling swab cryovials with

A

Swab Extraction-free	CDC Protocol Extraction +	CDC Protocol Extraction –	
+	38	0	38
–	0	31	31
	38	31	69

PPA = 100.0 %
NPA = 100.0 %

B

Saliva Extraction-free	CDC Protocol Extraction +	CDC Protocol Extraction -	
+	81	1	82
-	1	170	171
	82	171	253

PPA = 98.8 %
NPA = 99.4 %

Figure 6: Comparison of extraction-free RT-qPCR to the CDC assay. Extraction-free RT-qPCR and the CDC assay were performed on clinical **(A)** nasal swab and **(B)** saliva samples. PPA, positive percent agreement; NPA, negative percent agreement.

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VTB. Patients were given the choice to provide a swab or saliva sample, and the overwhelming majority opted for anterior nares swab (83.6%) compared to saliva (16.4%). Preparation of more swab kits (pre-filled with VTB) than saliva kits (empty) represented a significant challenge to the laboratory. The frequency of SARS-CoV-2 detection was 7.7% in swab samples and 10.1% in saliva (Figure 8).

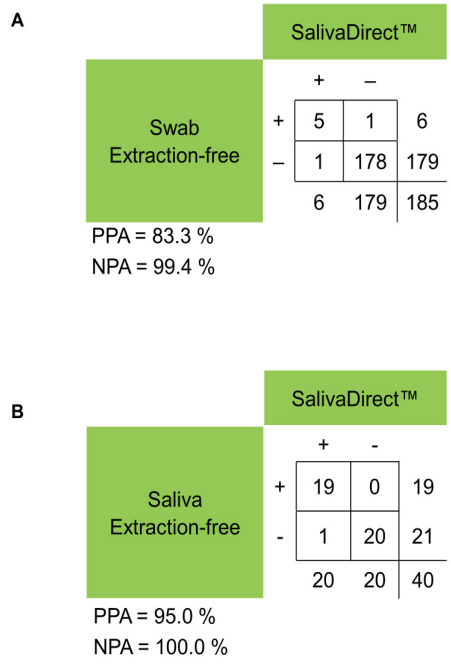

Figure 7: Comparison of extraction-free RT-qPCR to SalivaDirect™. Extraction-free RT-qPCR and SalivaDirect™ were performed on clinical **(A)** nasal swab and **(B)** saliva samples. PPA, positive percent agreement; NPA, negative percent agreement.

	Swab	Saliva	Total
SARS-CoV-2 samples (#)	26,425	6,775	33,200
Total samples	342,734	67,149	409,883
SARS-CoV-2 samples (%)	7.7%	10.1%	8.1%

Figure 8: Summary of clinical testing results. Extraction-free RT-qPCR testing of 410,000 samples over 10 months was performed. The total number of samples, the number of samples with detectable SARS-CoV-2, and the rate of SARS-CoV-2 detection for each sample are reported.

Discussion

The extraction step in conventional RT-qPCR is laborious, expensive, and dependent on the commercial supply chain. We describe a high-throughput, semi-automated, extraction-free laboratory developed RT-qPCR test for detection of SARS-CoV-2 in swab and saliva. Bypassing nucleic acid extraction saved resources, streamlined laboratory operations, and maximized technologist efforts to deliver results to patients. Although extraction-free RT-qPCR testing of saliva for SARS-CoV-2 has previously been reported [16-21], a standardized container and extraction-free process for testing of both swab and saliva samples on an integrated platform provides unique flexibility and resilience. To accomplish this, a panel of buffers was evaluated to formulate an optimal solution for each swab and saliva samples. A post-collection TCEP-based buffer was validated for saliva. A TBE-based viral transport buffer (VTB) for nasal swabs served dual purposes: pre-analytical sample stability and matrix compatibility with extraction-free testing. Although collection kits differed based on cryovial preparation and inclusion of a swab or a saliva collection device, use of the same cryovial with an externally threaded screw cap was a key feature to facilitate high-throughput testing using semi-automatic decapping machines. Without the burden of nucleic acid extraction, laboratory staff were able to prepare nearly 410,000 collection kits and test as many samples in 10 months. This advantage became essential since more than 5 patients opted for nasal swab collection for every one patient who provided saliva. In other words, laboratory staff pre-filled approximately 343,000 nasal swab cryovials with VTB and approximately 67,000 saliva collection kits with empty cryovials, despite the convenience and efficiency of the latter. The reasons for nasal swab preference are unclear but may be related to perceived convenience of sample collection, false belief that nasal swab is superior to saliva, or difficulty generating saliva. In addition, saliva collection was excluded for individuals who recently consumed food, beverages, or tobacco or used oral hygiene products or other potential interfering substances [8]. Interestingly, the SARS-CoV-2 detection rate was higher in saliva compared to nasal swab. Since these data are from real world experience, this finding does *not* suggest that clinical sensitivity for COVID-19 is higher using saliva than nasal swab. Instead, it may be related to non-random patient sampling or inconsistent distribution of sample types throughout waves of the pandemic. Testing of swab and saliva samples by the same high-throughput, extraction-free RT-qPCR platform is a valuable tool for surveillance and diagnosis of upper respiratory infectious disease, although equivalent clinical sensitivity of saliva compared to nasal swab would need to be demonstrated for each pathogen. This model is a scalable and cost-effective approach pandemic preparedness planning.

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Author contributions

Conceptualization (YQ, BB, XY, JZ, SL, BH)

Investigation (YQ, LL, AH, RT, SY, FG, AL, XY, SL)

Methodology (YQ, LL, XY, SL, BH)

Data Curation (YQ, LL AH, RT, SY, FG, AL, XY, SL)

Formal Analysis (DG, SL, BH)

Writing – original draft (XY)

Writing – review & editing (XY, SL, BH)

Resources (SL)

Funding acquisition (SL)

Competing interests

DG, BB, XY, JZ, BH, and SL previously owned equity in Summit Biolabs, Inc. (Aurora, CO).

References

1. J Peto, et al. Universal weekly testing as the UK COVID-19 lockdown exit strategy. Lancet 395 (2020): 1420-1421.
2. L Falzone, et al. Sensitivity assessment of droplet digital PCR for SARS-CoV-2 detection. Int J Mol Med 46 (2020): 957-964.
3. MJ Mina, KG Andersen. COVID-19 testing: One size does not fit all. Science 371 (2021): 126-127.
4. DB Larremore, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv 7 (2021).
5. MJ Mina, R Parker, DB Larremore. Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. N Engl J Med 383 (2020): e120.
6. GB Barra, TH Santa Rita, PG Mesquita, et al. Analytical Sensitivity and Specificity of Two RT-qPCR Protocols for SARS-CoV-2 Detection Performed in an Automated Workflow. Genes (Basel) 11 (2020).
7. W Wang, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 323 (2020): 1843-1844.
8. NE Babady, et al. Performance of Severe Acute Respiratory Syndrome Coronavirus 2 Real-Time RT-PCR Tests on Oral Rinses and Saliva Samples. J Mol Diagn 23 (2021): 3-9.
9. YP Tu, et al. Swabs Collected by Patients or Health Care Workers for SARS-CoV-2 Testing. N Engl J Med 383 (2020): 494-496.
10. AL Wyllie, et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. N Engl J Med 383 (2020): 1283-1286.
11. GB Barra, TH Santa Rita, PG Mesquita, et al. Overcoming Supply Shortage for SARS-CoV-2 Detection by RT-qPCR. Genes (Basel) 12 (2021).
12. MN Esbin, et al. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. RNA 26 (2020): 771-783.
13. V Genoud, et al. Extraction-free protocol combining proteinase K and heat inactivation for detection of SARS-CoV-2 by RT-qPCR. PLoS One 16 (2021): e0247792.
14. CBF Vogels, et al. SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. Med 2 (2021): 263-280 e266.
15. CDC, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.
16. CDC. in https://www.fda.gov/media/134922/download. (2020).
17. SA Byrnes, et al. Multiplexed and Extraction-Free Amplification for Simplified SARS-CoV-2 RT-PCR Tests. Anal Chem 93 (2021): 4160-4165.
18. N Lubke, et al. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. J Clin Virol 130 (2020): 104579.
19. N Merindol, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J Clin Virol 128 (2020): 104423.
20. I Smyrlaki, et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun 11 (2020): 4812.
21. C Batejat, Q Grassin, JC Manuguerra, et al. Heat inactivation of the severe acute respiratory syndrome coronavirus 2. J Biosaf Biosecur 3 (2021): 1-3.