Saline collection media and an extraction free workflow enables massively scalable, highly sensitive, and cost-effective SARS-CoV-2 testing

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Method Article

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Abstract

The challenges in scaling up SARS-CoV-2 testing capacity include shortages in the supply chain for consumables and reagents. Improvements in consumption patterns can be obtained through removal of key processing steps, including RNA extraction. Here, we present a scalable and validated extraction-free method for the detection of SARS-CoV-2 from swab specimens in saline, with a limit of detection at 1,000 GCE/mL and a sensitivity and specificity of 100%.

Main Text

With sufficient data that suggest SARS-CoV-2 can be spread by pre-symptomatic/asymptomatic carriers[1], large-scale and repeated testing for SARS-CoV-2 infection is critical to control the spread of COVID-19[2]. The standard workflow often includes total RNA extraction followed by real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). One of the challenges in scaling up SARS-CoV-2 testing capacity includes shortages in the supply chain for consumables and reagents. Therefore, reduced consumption can be achieved through removal of key processing steps such as RNA extraction.

An extraction-free workflow is less laborious than a RNA extraction workflow, but it is also less robust and prone to failure due to reaction inhibition by specimen components[3]. Studies suggested that dry swabs [4,5] and swab samples stored in appropriate transport media such as Universal/Viral Transport Media (UTM/VTM) [3,5–10] or water [6] can be tested by RT-qPCR without the need for RNA extraction. The general workflow includes a lysis step, incubating samples between 70°C to 99°C for 5-15 minutes, followed by RT-qPCR setup using a small amount of sample and specific RT-qPCR mastermixes. Only one of these studies[8] reported a limit of detection (LoD), which is greater than 6,000 genomic copies equivalent/mL (GCE/mL) and it was high for a PCR-based assay [11]. In addition, the increased demand for testing has constrained the supply of UTM/VTM. Alternative transport media and, at the very least, an end-to-end protocol with comparable sensitivity to extraction-based methods is essential to alleviating possible trade-offs between efficiency and sensitivity.

Saline is easily obtainable and most importantly has demonstrated stability and usability in swab-based sampling [12]. Saline transport media is a mixture of salt (sodium chloride) and water at a pH of 4.5-7.0, which is similar to the sodium concentration of human bodily fluids. Saline has been shown to perform poorly in an extraction-free workflow when the sample is added directly into the RT-qPCR reaction [3,6]. However, the workflow described here was adapted from a saliva-based SARS-CoV-2 molecular test [8] at which the sample is first diluted with 1X TBE ((Tris/Borate/EDTA Buffer) and then treated with heat at 95°C for 15 minutes. The heating step lysed the viral particles, and is also likely to inactivate the

inhibitory components in the sample, therefore allowing better sensitivity in detecting SARS-CoV-2 nucleic acid. The method is further optimized to be used for processing saline-based swab specimens.

To establish the limit of detection (LoD) of the assay, contrived samples were generated from upper respiratory specimens negative for SARS-CoV-2 collected via anterior nares swab in 0.85% saline solution. The samples were pooled to obtain a large volume of negative matrix and spiked with gamma-irradiated 2019 SARS-CoV-2 virus (BEI Resources) at an appropriate concentration. Triplicates were screened at each concentration of inactive virus, ranging from 4,000 GCE/mL to 250 GCE/mL (Figure 1). The final LoD was confirmed using 20 replicates. All three replicates were called positive at 500 GCE/mL during LoD screening; however, only 16/20 samples were called positive at 500 GCE/mL during confirmation. The extraction-free LoD was confirmed at 1,000 GCE/mL with 20/20 positive samples.

To evaluate the performance of extraction-free assay on clinical samples, this study used 30 positive and 30 negative remnant clinical swab samples in saline provided by an independent clinical lab. The samples were processed through the Helix standard extraction workflow as well as the extraction-free workflow. Cycle quantification (Cq) was used to measure viral load and guide the qualitative interpretation of samples. The extraction-free workflow achieved 100% concordance with clinical samples tested with extraction workflow. However, the median Cq of the extraction-free workflow was ~3-4 Cq higher compared to the extraction workflow (Figure 2a). There is a linear correlation of the Cq between the extraction and EF workflows with the exception of two low positive samples on N gene amplification (Figure 2b).

Here, we presented a massively scalable, highly sensitive and cost effective method in detecting SARS-CoV-2 for SARS-CoV-2 testing using saline collection media. The method outlined here consists of a minimal number of steps and utilizes a standard qPCR assay downstream, thus allowing direct implementation into the existing workflow. Saline has demonstrated usability in swab-based sampling [12] and is easily obtainable. Saline can be stored at room temperature. Upon sample collection, the sample is stable for up to 54 hours without special storage conditions [12]. However, saline-based specimens have been shown to perform poorly in extraction-free workflow [3,6]. In contrast, the method presented here utilizes TBE dilution, heat treatment, and large sample input volumes to achieve 100% sensitivity and specificity in 60 clinical samples, and a LoD at 1,000 GCE/mL, which is comparable to assays using extraction-based methods [11,13]. The limitations of this study include the relatively small number of available clinical samples that preclude a more thorough analysis of sensitivity compared to extraction-based workflows. Even though the Cqs from the extraction-free workflow are higher than using extracted RNA, the LoD of the extraction-free workflow is equivalent, and detection of SARS-CoV-2 in clinical samples is highly correlated between the two methods. Our results suggest that a properly validated

extraction-free RT-qPCR workflow can achieve the level of accuracy and sensitivity needed for reliable detection of SARS-CoV-2 in clinical samples. The extraction-free workflow using saline transport media removes supply chain constraints, has high accuracy and sensitivity, and it is simple, cost effective and massively scalable.

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Declarations

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Conflict of Interest Disclosures

YWL, NAL, AJA, HM, NB, JTL, DB are employees of Helix

Online Only Methods

Specimen Collection Materials

Oropharyngeal Flocked Swab (Affordable IHI) was used in this study. 0.85%-0.90% saline was used as transport media.

Sample Preparation for Limit of Detection and Confirmation Studies

Contrived samples were generated from upper respiratory specimens negative for SARS-CoV-2 collected via anterior nares swab in 0.85% saline solution. The samples were pooled to obtain a large volume of negative matrix. Gamma-irradiated 2019 SARS-CoV-2 virus (BEI Resources) was then spiked into the negative matrix at an appropriate concentration and treated at 65°C for 10 minutes. After the deactivation step, 50 µl of the contrived sample was added to 2X volume (100 µl) of 1X TBE buffer (method adopted from Ranoa *et. al.* [14]) with 1:200 diluted MS2 internal control (Thermo Fisher, PN A47814), corresponding to a final MS2 dilution of 1:300 from original stock. Diluted samples were treated at 95°C for 15 minutes prior to RT-qPCR.

Sample preparation for clinical samples

A total of 30 positive for SARS-CoV-2 and 30 negative clinical remnant samples derived from anterior nares swabs collected in normal saline were provided by an independent clinical lab (Laboratory Corporation of America). The samples were stored at -80°C upon initial processing, and were shipped in dry ice to the Helix laboratory. Samples were heat-inactivated at 65°C for 10 min upon receiving. The samples were processed through the Helix COVID-19 Test (EUA2016360), as well as the extraction-free (EF) workflow. A total of 50 µl of each sample was added to 100 µl of 1X TBE with MS2 internal control

(1:300 MS2 final dilution of original stock). For each sample, 14.25 µl was transferred to a new Hard-Shell 384-well PCR plate for lysis at 95°C for 15 minutes. Per sample, 5 µl of One-step RT-PCR mastermix MDX016 (Meridian BioScience) and 0.75 µl of primer/probe mix from TaqPath™ COVID-19 Combo Kit (Thermo Fisher) was added directly to the lysed samples, followed by RT-qPCR at a 20 µl total reaction volume.

RT-PCR setup for extraction-free workflow

The RT-qPCR assay was set up using the TaqPath™ COVID-19 Combo Kit (Thermo Fisher) from Thermo Fisher (EUA200010) and Inhibitor Tolerant 1-Step RT-qPCR Mastermix MDX016 (Meridian BioScience). The cycling conditions are shown in Table 1. The RT-qPCR assay was carried out with the QuantStudio™ 7 Flex Real-Time PCR System.

Table 1: Temperature and cycling conditions for Helix's extraction-free workflow using Meridian MDX016 mastermix.

Meridian MDX016 One Step RT qPCR 4X Mastermix	Temperature	Time	Cycles
RT reaction	52°C	20 min	1
Enzyme activation	95°C	2 min	1
Denature	95°C	5 sec	45
*Anneal/Extend (*Read)	62°C	60 sec	

Interpretation of Sample based on the number of targets amplified

The targeted gene is considered positive if the amplification curve crosses the threshold line within 39 quantification cycles (Cq < 39) and has a Cq confidence score > 0.8. The final outcome of the individual sample is based on the number of targets detected as shown in Table 2. Cycle quantification (Cq) was used for qualitative interpretation of samples. True positives (TP) were defined by test results generated using the Helix COVID-19 Test. Sensitivity was defined as (TP)/(TP+FN) and specificity was defined as (TN)/(TN+FP).

Table 2: Interpretation of the RT-qPCR results per sample.

ORF1ab	N gene	S gene	MS2	Status	Results
NEG	NEG	NEG	NEG	Invalid	NA
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 not detected
NEG	NEG	POS with Cq >37*	POS	Valid	SARS-CoV-2 not detected
Only one SARS-CoV-2 target = POS			POS or NEG	Valid	SARS-CoV-2 Inconclusive
Two or more SARS-CoV-2 targets = POS			POS or NEG	Valid	Positive for SARS-CoV-2

***** Criteria to exclude artifacts introduced by low level S gene amplification in negative samples, POS with Cq >37 and Cq confidence score >0.8.

Statistical analysis

Differences between extraction and extraction-free Cqs of the clinical positive samples were analyzed using a paired t-test. Correlations between the clinical positive samples extraction and extraction-free Cqs per gene target were analyzed using Spearman's rank test. Analyses were performed with GraphPad Prism.

Figures

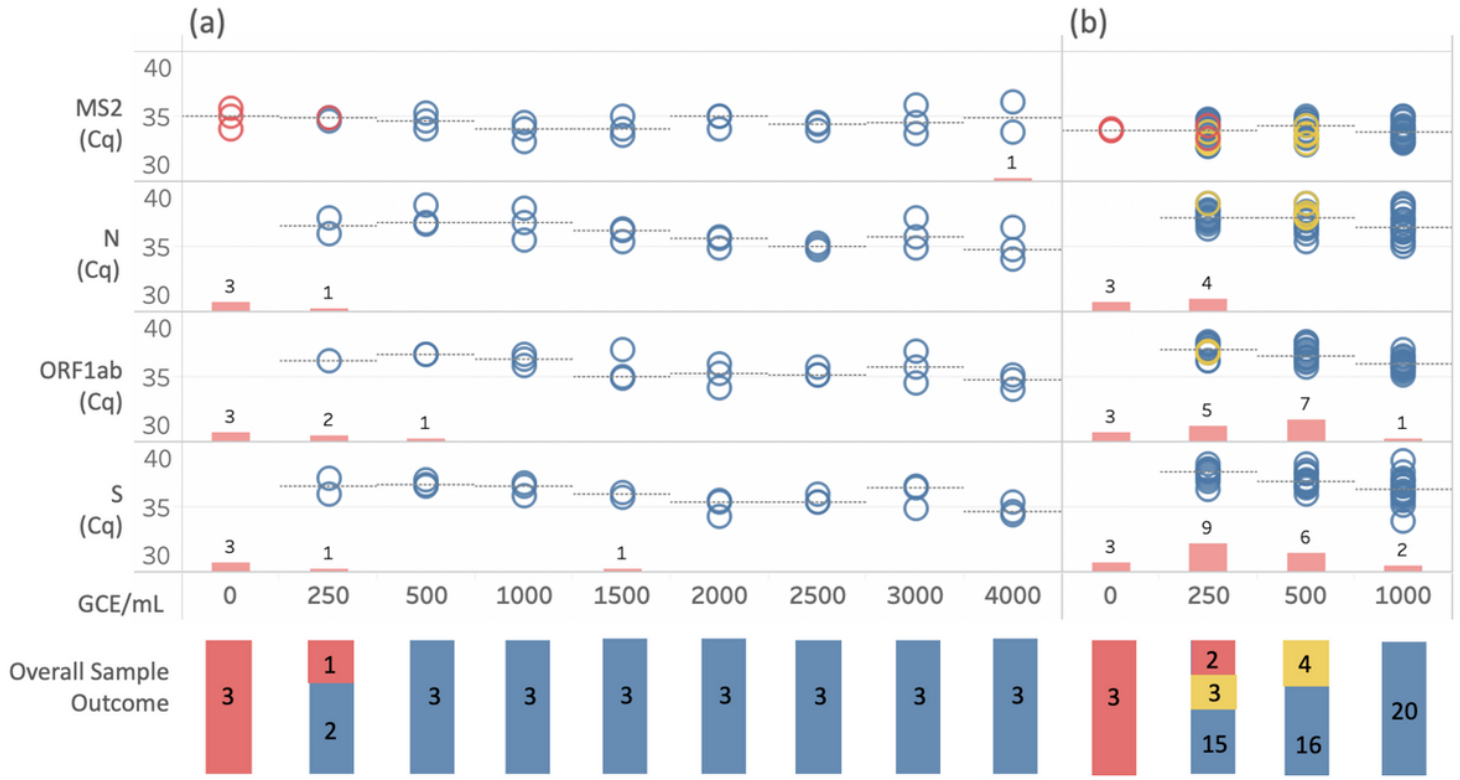

Figure 1

LoD of SARS-CoV-2 from anterior nares swab in saline solution. Gamma-irradiated SARS-CoV-2 was spiked into SARS-CoV-2 negative matrix. Specimens were mixed at 1:2 sample to TBE (Tris/Borate/EDTA Buffer) ratio, and were treated at 95°C for 15 min prior to RT-qPCR. All samples were analyzed in triplicate for LoD screening (a) and 20 replicates for LoD confirmation (b). Total non-amplified targeted calls are shown as pink bars. The LoD was confirmed by the amplification of 20/20 replicates at 1,000 GCE/mL. Blue = Positive, Yellow = Inconclusive, Red = Not Detected.

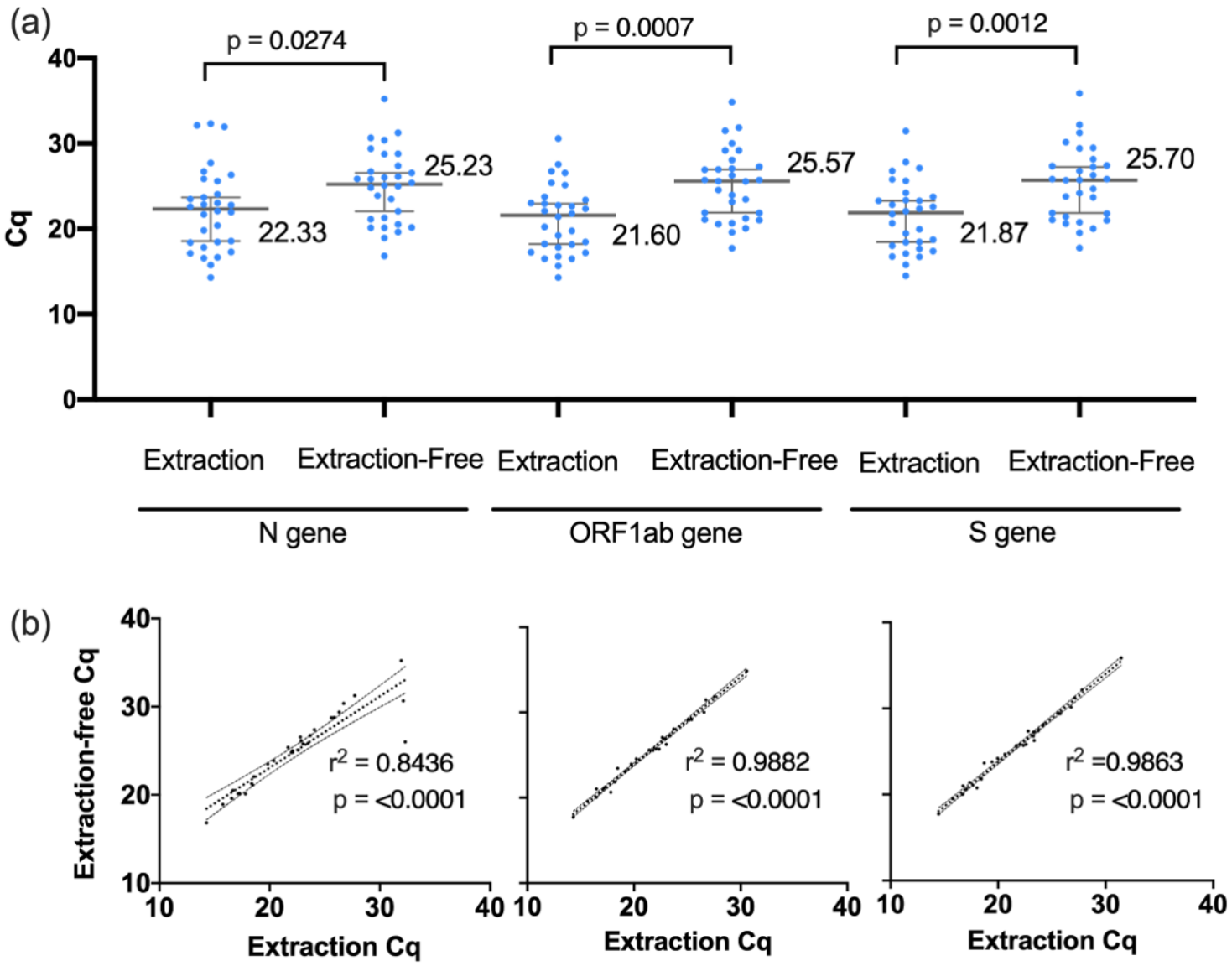

Figure 2

Comparison of Cq between extraction and extraction-free workflow across the same SARS-CoV-2 positive clinical samples. Distribution of Cq (a) and pairwise correlation (b) of Cq across the samples processed with extraction and extraction-free workflow.