

*Protocol*

# **A 5-min RNA preparation method for COVID-19 detection with RT-qPCR**

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**\*\*\* IMPORTANT NOTE: This protocol has NOT been approved for clinical use.** We only have access to a very limited number of patient samples and as a result have not been able to validate this assay extensively using a large number of patient samples.

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## **Abstract**

Current wide-spread clinical testing for the novel coronavirus SARS-CoV-2, including the CDC test, requires extraction of RNA from patient samples followed by RT-qPCR. The initial RNA extraction step adds complexity to the overall diagnostic and relies on reagents that are in short supply. We report here a protocol for an alternative sample processing step that takes only five minutes and does not require RNA extraction. We demonstrate (on a limited number of samples) that this protocol is compatible with nasopharyngeal swab sample inputs and does not impact RT-qPCR sensitivity.

## **Introduction**

The novel coronavirus SARS-CoV-2 causes COVID-19 and has resulted in an international public health emergency, spreading to over 180 countries and infecting more than a million individuals. Testing for the presence of the virus is of utmost importance to both reduce the basic reproductive rate of the virus ( $R_0$ ) and inform best clinical practices for affected patients. However, understanding the full extent of the virus outbreak has remained challenging due to bottlenecks in the diagnosis of infection.

Currently, the majority of testing is being performed using RT-qPCR, although serological tests are coming into wide use. For early detection of infection, however, PCR-based methods will continue to be essential. Although several variations of PCR-based tests (as well as isothermal amplification based methods) are being clinically used (<https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>), typically they all require an RNA extraction step prior to detection.

RNA extraction has become a major bottleneck for COVID-19 diagnosis due to the limited availability of RNA extraction kits for preparing virus RNA from patient samples and the low-throughput nature of the extraction procedure. Here, we describe a one-step column-free RNA preparation method that can be carried out in 5 minutes. The reaction can be used directly with

the CDC COVID-19 RT-qPCR testing protocol, thus increasing throughput and alleviating supply chain issues.

## **Materials and Methods**

### *Reagents*

- Quick Extract™ DNA Extraction Solution ([QE09050](#)), Lucigen. Once thawed, aliquot and store at -20C to avoid >3 freeze-thaw cycles.
- Nasopharyngeal swab stored in Viral Transport Medium

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1. Dilute nasopharyngeal swab stored in Viral Transport Medium or Human Specimen Control (HSC) 1:1 with Quick Extract™ DNA Extraction Solution. For example, in a fresh PCR tube, mix 20 µl of swab sample with 20 µl of Quick Extract.
2. Incubate swab and Quick Extract mix at **95°C for 5 minutes**. Allow reaction to cool on ice before proceeding.
3. Use reaction from step (2) for qRT-PCR. Use an amount from step (2) that corresponds to 10 % of the total qRT-PCR reaction volume. For example, for a reaction with total volume of 50 µl, use 5 µl of the reaction from step (2).

## **Results**

We evaluated a number of buffer compositions to identify one that achieved efficient lysis of enveloped virus while preserving the activity of the CDC recommended RT-qPCR reaction (TaqPath™ 1-Step RT-qPCR Master Mix). Of all of the buffers tested, Quick Extract™ DNA Extraction Solution provided results that were comparable to standard RNA extraction kits.

To confirm that the presence of QE does not interfere with RT-qPCR activity, we compared RT-qPCR reactions using synthetic SARS-CoV-2 gene fragment ([Twist Synthetic SARS-CoV-2 RNA Control 1, SKU:102019](#)) dissolved in either ddH<sub>2</sub>O or in a 50:50 ddH<sub>2</sub>O:Quick Extract

mixture. We set up each RT-qPCR reaction with a total volume of 10  $\mu$ l (1  $\mu$ l of RNA sample, 0.5  $\mu$ l of CDC probe N1, 2.5  $\mu$ l of TaqPath RT-qPCR master mix, and 6  $\mu$ l of ddH<sub>2</sub>O). From these reactions, we found that Quick Extract at a final concentration of 5 % did not negatively affect the RT-qPCR reaction (Figure 1A; 2 technical replicates).

We conducted preliminary validation of the Quick Extract RNA preparation procedure on COVID-19 positive nasopharyngeal swabs and found that RNA samples prepared using Quick Extract supported similarly sensitive detection of coronavirus as QIAmp Viral RNA Miniprep (Qiagen) for all 4 swab samples (Figure 1B; 2 biological replicates). To simulate low viral load, coronavirus positive swabs were diluted 1:10 in pooled nasopharyngeal swabs from 5 healthy donors ([Lee Biosolutions, SKU:991-31-NC-5](#)) prior to purification or Quick Extract treatment. For the QIAmp Viral RNA Miniprep conditions, 100  $\mu$ L of diluted swab sample was used for extraction and was eluted using 100  $\mu$ L of ddH<sub>2</sub>O. 1  $\mu$ L of the elution was used in a 10  $\mu$ L RT-qPCR reaction. For the Quick Extract conditions, 1  $\mu$ L of Quick Extract preparation was used for each 10  $\mu$ L RT-qPCR reaction.

## **Conclusion**

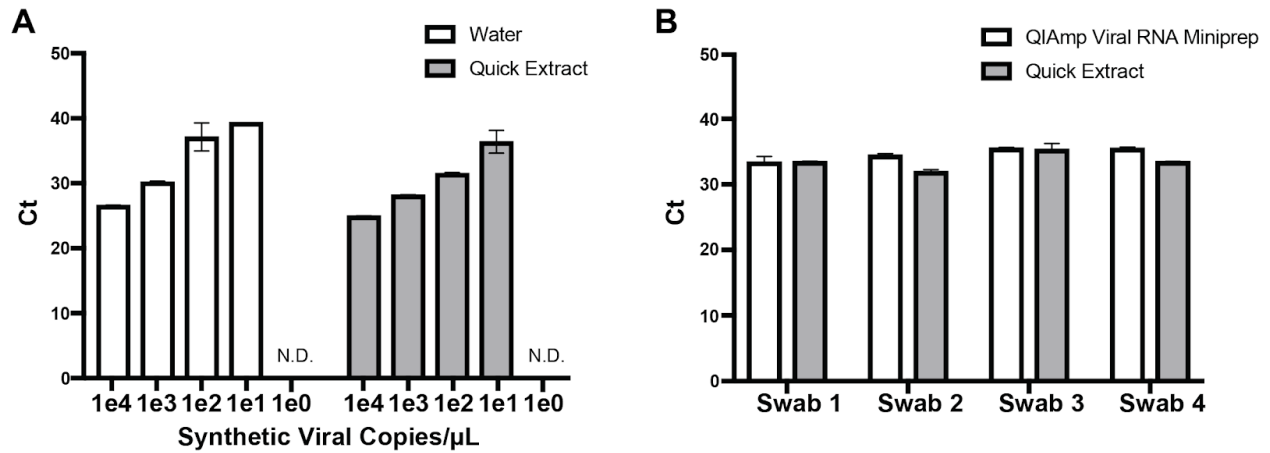
We present here an alternative sample extraction method suitable for use with RT-qPCR-based detection of COVID-19 that does not rely on RNA extraction kits. By testing a number of buffers, we found that a 5-minute approach using Quick Extract performs similarly to FDA-approved RNA extraction kits. We note that this protocol has only been validated on a small number of clinical samples, and therefore should be more extensively tested and vetted by appropriate regulatory agencies before being used in a clinical setting.

**Declaration of Interests:** O.O.A., and J.S.G. are co-founders, scientific advisors, and hold equity interests in Sherlock Biosciences, Inc. F.Z. is a co-founder of Editas Medicine, Beam Therapeutics, Pairwise Plants, Arbor Biotechnologies, and Sherlock Biosciences.

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Institute; the Howard Hughes Medical Institute; Open Philanthropy Project; James and Patricia Poitras; and Robert Metcalfe.

**Ethical Statement:** Patient samples were existing biospecimens and de-identified. This work was determined to be “not human subjects research”.



**Figure 1. A)** RT-qPCR of synthetic SARS-CoV-2 RNA control ([Twist Synthetic SARS-CoV-2 RNA Control 1, SKU:102019](#)) diluted in water or 50:50 ddH<sub>2</sub>O:Quick Extract mixture. Final sample volume was 1  $\mu$ L in a 10  $\mu$ L reaction. **B)** RT-qPCR of diluted COVID-19 positive nasopharyngeal swabs treated at 95°C for 5 minutes in Quick Extract or purified using QIAmp Viral RNA Miniprep.