

Short communication

Use of a simplified sample processing step without RNA extraction for direct SARS-CoV-2 RT-PCR detection



Ruby Barza, Parul Patel, Linda Sabatini, Kamaljit Singh *

NorthShore University HealthSystem, Department of Pathology and Laboratory Medicine, Evanston, IL, USA

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ABSTRACT

The severe acute respiratory syndrome coronavirus (SARS-CoV-2) pandemic has resulted in significant shortages of RT-PCR testing supplies including RNA extraction kits. The goal of our study was to determine if a simplified heat-RNA release method would provide comparable detection of SARS-CoV-2 without the need for nucleic acid extraction. RT-PCR results using the ChromaCode HDPCR™ SARS-CoV-2 were compared using the heat-RNA release method and an automated RNA extraction system (EMAG). The heat-RNA release method correctly identified 94 % (81/86 nasopharyngeal samples) that were positive for SARS-CoV-2. Five samples that were missed by heat-RNA release method had a mean Ct value: 35 using the automated extraction instrument, indicating a very low viral load. Our findings show that a simple heat-RNA release method is a reasonable alternative for the majority of COVID-19 positive patients and can help overcome the cost and availability issues of RNA extraction reagents.

1. Introduction

The severe acute respiratory syndrome coronavirus (SARS-CoV-2), continues to be a growing worldwide public health concern and the expansion of diagnostic testing is considered a critical requirement for case detection, contact tracing and control of the spread of COVID-19 infection [1]. Shortages of test materials have resulted in a forced narrow testing strategy in the United States dedicated to managing the care of the sickest patients that require hospitalization, thereby hampering efforts to identify and prevent community transmission of COVID-19 [2]. Of growing concern is the expansion of the pandemic in low- to middle-income countries which already lack testing materials and the ability to compete with powerful developed countries to procure test reagents for their population [3]. The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) both recommend standardized SARS-CoV-2 molecular diagnostic protocols that include an RNA extraction step from an upper respiratory sample followed by reverse transcription polymerase chain reaction (RT-PCR) to detect the purified SARS-CoV-2 RNA [4]. However, recent shortages of testing materials, particularly shortages of RNA extraction reagents, poses a major obstacle.

A potential solution during a period of extraction reagent shortages

might be to use a simple heat inactivation and extraction step as an alternative to automated RNA extraction systems or manual kits which are also more expensive and time and labor intensive [4]. The destabilization of the physical integrity of viruses by heating results in the release of viral RNA which is then available for a RT-PCR detection. However, the temperature at which virus particles disintegrate during heating differs significantly between virus types and physicochemical conditions [5]. To improve the real-time RT-PCR detection of SARS-CoV-2 RNA, a heat release and inactivation step was evaluated. Our goal was to simplify the pre-PCR step and demonstrate that a simple heat-RNA release step can be an alternative to the traditional RNA extraction approach. We compared the diagnostic sensitivity of the heat-RNA release method for detection of SARS-CoV-2 using NP swabs in viral transport media (VTM) to results obtained using an automated RNA extraction system (EMAG®, bioMérieux, Durham, NC).

2. Methods

A total of 174 COVID-19 positive samples were used for the study comprising 87 unique COVID-19 NP patient swabs in VTM (BD, Sparks, MD). For automated RNA extraction, 500 µL of VTM was used on the EMAG® with a final eluate volume of 50 µL representing a 10-fold

* Corresponding author at: NorthShore University HealthSystem, Department of Medicine, Division of Infectious Diseases, Burch Room 124, 2650 Ridge Avenue, Evanston, IL, 60201, USA.

E-mail address: ksingh@northshore.org (K. Singh).

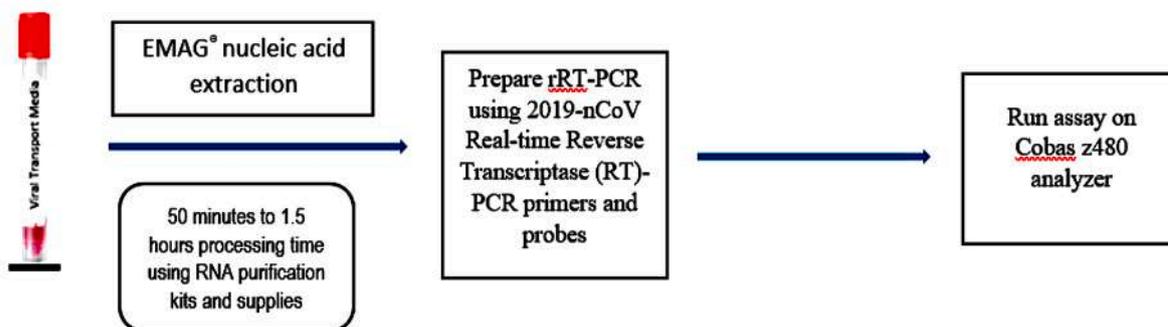
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Current protocol:



Modified protocol using heat-RNA release method:

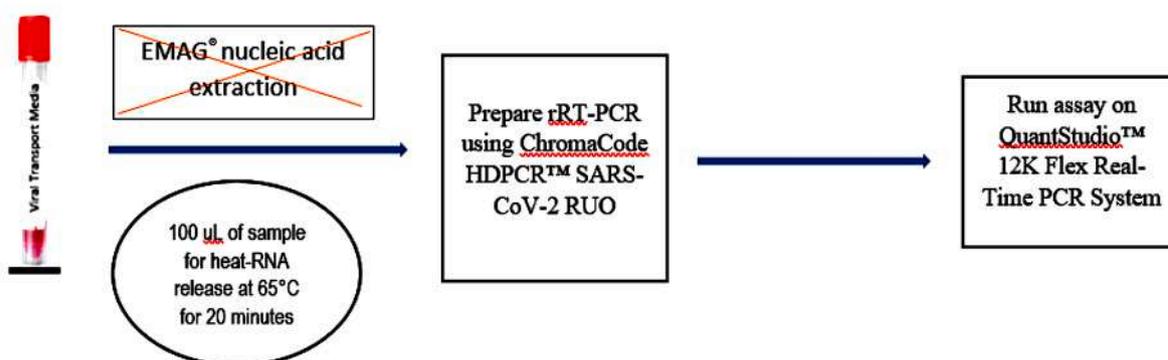


Fig. 1. Current protocol and modified protocol using heat-RNA release method.

concentration. The extracted RNA samples were initially tested using a laboratory developed test (LDT) based on the CDC primer-probe sets for 2019-nCoV_N1 and 2019-nCoV_N2 and analyzed using the Cobas z480 instrument (Roche, Indianapolis, IN). The SARS-CoV-2 RNA copy levels using the LDT were previously determined using standardized RNA controls and included an RNA extraction step using the EMAG®. The final Ct values for all positive samples were recorded and the samples were divided into 3 different categories for the study with an equal number (29 samples each) with Ct values of (a) <20, (b) 20–30 and (c) >30.

For the heat-RNA release method, 100 µL of VTM was heated to 65 °C for 20 min and spun down for 30 s [5,6]. A 5 uL final volume was used for SARS-CoV-2 RT-PCR testing with the ChromaCode HDPCR™ SARS-CoV-2 Research Use Only (ChromaCode RUO) assay (Fig. 1). The ChromaCode RUO assay also utilizes the CDC primer-probe sets for 2019-nCoV_N1 and 2019-nCoV_N2. A 5 µL volume of extracted RNA using the EMAG® was tested in parallel using the ChromaCode RUO assay. The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher, USA) was used for SARS-CoV-2 RT-PCR analysis. RT-PCR results, including Ct values using the heat-RNA release method were compared to the results obtained using the automated RNA-extraction method with both the LDT and ChromaCode SARS-CoV-2 assays.

3. Results

Both the Chromacode SARS-CoV-2 RUO and the in-house LDT assay showed good agreement using the two different extraction methods, 99 % (86/87 samples) with the EMAG® automated extraction and 93 % (81/87 samples) for the heat-RNA release method. Comparison of the

Table 1
Comparison of Ct difference between heat-RNA release and EMAG® for 5 Negative samples.

Sample Number	ChromaCode HDPCR™ SARS-CoV-2		LDT with EMAG®.
	EMAG® extraction	Heat inactivation	
1	35	No CT	36
33	33	No CT	35
46	35	62	36
56	37	No CT	38
86	34	86	36

heat-RNA release method to EMAG® using the ChromaCode SARS-CoV-2 RUO alone yielded a 94 % agreement (81/86 positive samples). We observed slightly higher Ct values using the heat-RNA release method but most of the observed Ct difference fell between <1 to 3 Ct values. Use of the heat-RNA release method yielded 100 % (29/29) agreement for samples with Ct value <20, 100 % (29/29) agreement for Ct values between 20–30 and 83 % (24/29) agreement for Ct values between 30–40. The five samples that were missed using the heat-RNA release method all had low amounts of RNA with a median Ct value 36 (Table 1). Of 675 cases with a positive SARS-CoV-2 diagnosis at our institution, only 8% would fall into this low copy range (Fig. 2).

4. Discussion

We found that the direct detection of SARS-CoV-2 using a 65 °C heat inactivation and release step for 20-minutes without RNA extraction and purification performed very well compared to the use of an automated RNA extraction instrument. Thermal pre-treatment is important as it

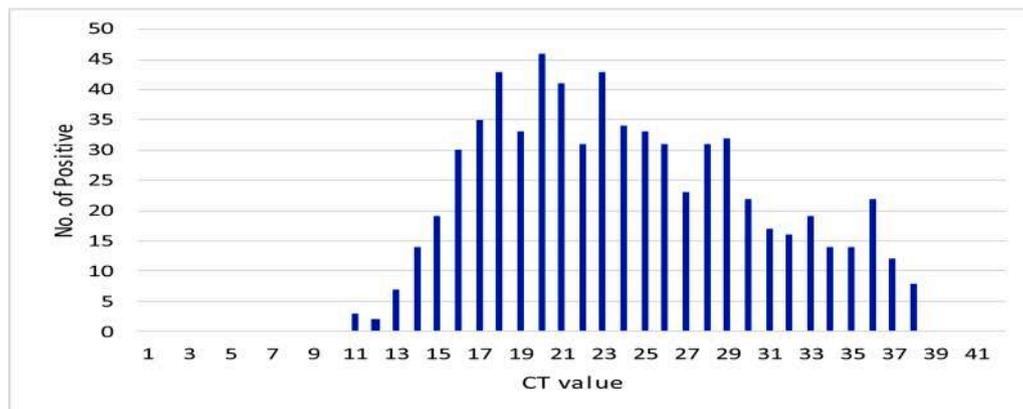


Fig. 2. CT values of confirmed 675 cases with positive SARS-CoV-2.

inactivates virus, causes exposure of viral genome and denatures inhibitors of the RT and/or PCR enzymes in the NP matrix. In contrast, the common practice of heating at high temperatures (95 °C for a few minutes) for direct RT-PCR in lieu of RNA extraction often results in lower RT-PCR sensitivity, presumably due to breakage of phosphodiester bonds within the targeted sequence [7].

We observed slightly higher Ct values using the heat-RNA release step but this is likely because RT-PCR performed with the automated extraction method uses a 10-fold higher concentration of the final eluate (500 µL VTM used for a final extraction volume of 50 µL). The concentration of final sample volume using the automated extraction method is also the likely explanation for the lower positive yield observed at Ct values ≥ 35 , which represents a very low viral load. As noted above, this low-copy range would only comprise 8% (56/675) of PCR positive samples in our laboratory. In conclusion, a substantial number of samples can be processed quickly without costly instrument and extraction reagent kits using a simple, calibrated heat inactivation and release method.

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Declaration of Competing Interest

The authors report no declarations of interest.

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ChromaCode HDP-PCR™ SARS-CoV-2 Research Use Only (ChromaCode RUO) assay kit.

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