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Evaluating the efficacy of RT-qPCR SARS-CoV-2 direct approaches in comparison to RNA extraction

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A B S T R A C T

The genetic identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is based on viral RNA extraction prior to RT-qPCR assay. However, recent studies have supported the elimination of the extraction step. This study was performed to assess the necessity for the RNA extraction, by comparing the efficacy of RT-qPCR in several direct approaches versus the gold standard RNA extraction, in the detection of SARS-CoV-2 in laboratory samples, as well as in clinical oro-nasopharyngeal SARS-CoV-2 swabs. The findings showed an advantage for the extraction procedure; however a direct no-buffer approach might be an alternative, since it identified more than 60% of positive clinical specimens.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in significant morbidity and mortality worldwide. At the time of writing, more than six million cases and over 370 000 deaths had been reported (Anon, 2020). The pandemic has created an acute need for rapid, cost-effective, and reliable diagnostic screening. The COVID-19 genetic diagnostics process includes RNA extraction from oro-nasopharyngeal swabs, followed by reverse transcriptase quantitative PCR (RT-qPCR) targeting viral genes (Corman et al., 2020). However, the global demand for reagents has placed extensive strain on supply chains for RT-qPCR kits, and to an even greater extent, on RNA isolation reagents. Potentially, eliminating RNA extraction would greatly simplify the diagnostic procedure, reducing both costs and the time to answer, while allowing testing to continue in the case of reagent shortages. Previous studies have demonstrated that several lysis buffers might allow the elimination of RNA extraction (Ladha et al., 2020; Pearson et al., 2020; Merindol et al., 2020). Very recently, two studies (Fomsgaard and Rosenstierne, 2020; Bruce et al., 2020) used a direct no-buffer RT-qPCR approach, which identified <90% of the tested clinical samples.

This study was performed to test the diagnostic efficiency following thermal inactivation (65 °C for 30 min and 95 °C for 10 min) without the addition of lysis buffer ('no buffer') or following lysis using one of three buffers (Virotype, QuickExtract, and 2% Triton-X-100), and to make a comparison to diagnosis after standard RNA extraction. Samples included buffers spiked with SARS-CoV-2 at concentrations of 0.1–100 000 plaque-forming units (PFU)/mL and 30 clinical samples, previously diagnosed as positive ($n = 20$) and negative ($n = 10$).

Methods

RNA standards and clinical samples

Viral RNA standards were viable SARS-CoV-2 (GISAID accession number **EPI_ISL_406862**), cultured in Vero E6 cells and diluted in viral transport medium (Biological Industries). Virus concentrations were recorded in PFU/mL; 1 PFU was determined as 1000 virions by digital PCR (data not shown). Oro-nasopharyngeal swab samples for the study were selected after approval by conventional RT-qPCR. Positive and negative samples were randomly selected for this study and kept at 4 °C until use.

RNA extraction

RNA was extracted from a 200-μl sample using an RNAdvance Viral Kit and the Biomek i7 Automated Workstation (Beckman Coulter) and eluted with 50 μL H_2O.
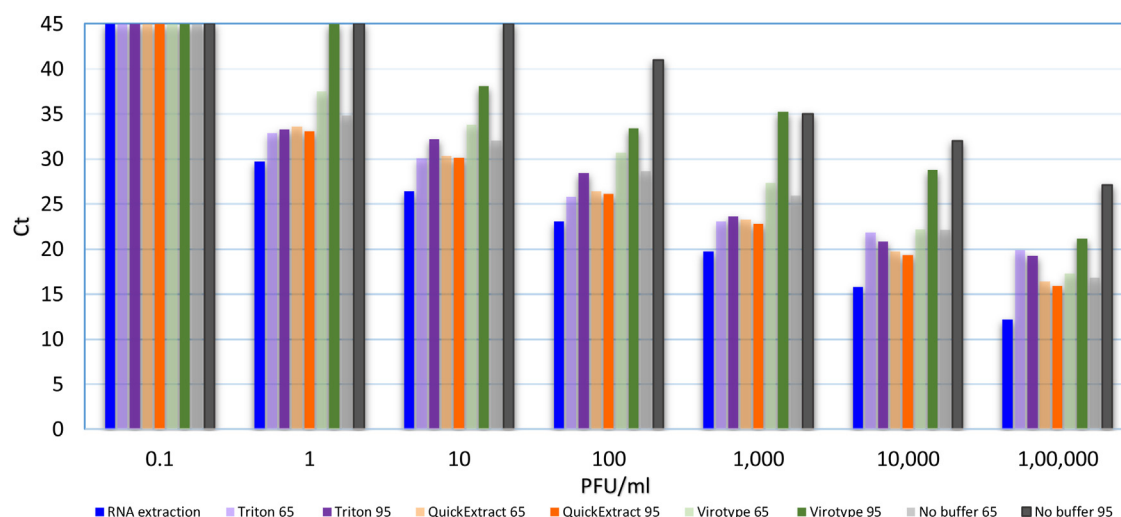
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Fig. 1. RT-qPCR results for the SARS-CoV-2 samples at different concentrations.
All of the samples were analyzed in duplicate. The average Ct values are shown. The different buffers and conditions are explained in the key at the bottom of the figure. Ct 45 = undetected; 65 = 65 °C, 95 = 95 °C.

Direct detection

Samples were analyzed directly or mixed 1:1 with one of the following buffers: QuickExtract DNA Extraction Solution (Lucigen), Virotype Tissue Lysis Reagent (Indical Bioscience GmbH), and 2% Triton-X-100 (Sigma) after inactivation at 95 °C for 10 min or 65 °C for 30 min.

RT-qPCR

The RT-qPCR assays were performed using the SensiFAST Probe Lo-ROX One-Step Kit (Bioline). Primers and probe for the SARS-CoV-2 E gene were taken from the Berlin protocol (Corman et al., 2020).

Results and discussion

SARS-CoV-2 samples in different concentrations

Standard samples were analyzed in duplicate. The results are shown in Fig. 1, as averages. The samples were analyzed following two inactivation temperatures: 95 °C for 10 min or 65 °C for 30 min. The maximum standard deviation was <2 Ct, with an average standard deviation of 0.4 across all samples. The limit of detection was 1 PFU/mL. At this concentration, samples in the no-buffer mode and Virotype at 95 °C were not detected, while the RNA extraction mode averaged the lowest critical threshold (Ct = 29.8), followed by QuickExtract and Triton. At 10 PFU/mL, only the no-buffer mode at 95 °C failed to detect. The RNA

Table 1
RT-qPCR results of SARS-CoV-2 clinical samples using different treatments; the Ct values are shown.

Patient number	RNA extraction	Triton-X-100, 2%				QuickExtract				Virotype				No buffer			
		65 °C	Δ	95 °C	Δ	65 °C	Δ	95 °C	Δ	65 °C	Δ	95 °C	Δ	65 °C	Δ	95 °C	Δ
1	17.0	31.9	15	31.3	14	18.6	1.5	20.4	3.4	23.7	6.6	24.2	7.1	18.3	1.2	21.9	4.8
2	19.5	U	–	U	–	24.4	4.9	25.4	5.9	27.9	8.4	24.9	5.5	25.4	5.9	22.7	3.2
3	21.7	U	–	U	–	31.4	9.8	30.9	9.3	33.8	12	30.5	8.9	28.5	6.8	27.9	6.3
4	28.8	U	–	U	–	33.7	4.9	35.6	6.9	35.3	6.5	33.1	4.3	31.8	3	31.7	3
5	29.0	U	–	U	–	33.7	4.7	35.4	6.4	38.7	9.8	35	6	32	3.1	32.5	3.6
6	29.6	U	–	U	–	34	4.4	U	–	35.6	6	U	–	31.4	1.8	29.7	0.1
7	30.3	U	–	U	–	U	–	U	–	U	–	U	–	39.1	8.8	35	4.7
8	30.3	U	–	U	–	U	–	U	–	U	–	U	–	U	–	38.8	8.6
9	31.2	U	–	U	–	36.2	5	U	–	U	–	U	–	35.2	4	35.3	4.1
10	31.4	U	–	U	–	U	–	42.4	11	U	–	38.4	7	U	–	36.1	4.8
11	31.6	U	–	U	–	U	–	U	–	U	–	U	–	U	–	35.8	4.2
12	32.0	U	–	U	–	U	–	U	–	U	–	U	–	U	–	37.6	5.6
13	32.4	U	–	U	–	U	–	37.5	5.1	35.7	3.3	38.6	6.2	35.6	3.2	36.9	4.5
14	32.9	U	–	U	–	U	–	U	–	U	–	U	–	38.3	5.4	U	
15	33.4	U	–	U	–	U	–	U	–	U	–	38.3	4.9	U	–	34.9	1.5
16	33.7	U	–	U	–	U	–	U	–	U	–	U	–	U	–	U	–
17	33.8	U	–	U	–	U	–	U	–	U	–	U	–	U	–	U	–
18	35.7	U	–	U	–	U	–	39.7	4	U	–	U	–	U	–	U	–
19	35.7	U	–	U	–	U	–	U	–	U	–	U	–	U	–	U	–
20	35.9	U	–	U	–	U	–	U	–	U	–	U	–	U	–	U	–
Detection level (%)[a]		5		5		35		40		35		40		50		70	

Ct, = cycle threshold; U = undetermined (Ct = 45); Δ = the difference in Ct between RNA extraction and the different treatments.
[a] Detection level = the percentage of positive samples (Ct <45).

extraction mode maintained the lowest Ct values across all of the concentrations analyzed. The minimum ΔCt average to the RNA extraction mode was obtained using QuickExtract, followed by Triton, Virotype, and the no-buffer mode.

SARS-CoV-2 clinical samples

Next we tested the feasibility of direct SARS-CoV-2 detection in clinical samples. Twenty positive and 10 negative samples were analyzed following thermal inactivation. All previously defined negative samples remained negative across the different buffers and test conditions. Positive samples exhibited major differences in detection capability (Table 1). The alternative buffers exhibited much lower detection levels: Triton (both inactivation protocols) detected a single positive sample (5% detection). QuickExtract and Virotype had 35–40% detection rates (both inactivation protocols). Surprisingly, the direct no-buffer approach was superior, with 50% detection for the 65 °C inactivation protocol and 70% for the 95 °C inactivation protocol. Detection was reversely correlated to the sample Ct value, with efficiency dropping from 100% for Ct $\leq$32 to 25% for samples with higher Ct. The 95 °C no-buffer approach was further evaluated in a larger set of known positive samples, producing similar results (total n = 43, 63% detection).

Conclusions

The results of this study demonstrate that RNA extraction significantly improves comprehensive and sensitive clinical diagnosis of SARS-CoV-2. We suggest that clinical samples, which include a multitude of nucleic acids and proteins, might significantly hamper detection. Although previously reported to facilitate viral detection (Ladha et al., 2020; Pearson et al., 2020; Merindol et al., 2020), the buffers tested here severely compromised the limit of detection (to a maximum of 40%). This is surprising, considering that direct analysis without adding buffers achieved a 63% detection level. This no-buffer direct approach could potentially be used with some success in times of need to achieve screening for high-titer samples.

Funding source

No funding source is applicable to this study.

Ethical approval

Pre-existing samples were used and de-identified. This work was therefore determined to be research not involving human subjects.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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