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Short Communication

Efficacy of a novel SARS-CoV-2 detection kit without RNA extraction and purification

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ABSTRACT

Rapid detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for the diagnosis of coronavirus disease 2019 (COVID-19) and preventing the spread of the virus. A novel detection kit – the 2019 Novel Coronavirus Detection Kit (nCoV-DK) – halves the detection time by eliminating the steps of RNA extraction and purification. We evaluated the concordance between the nCoV-DK and direct PCR. The virus was detected in 53/71 specimens (74.6%) by direct PCR and in 55/71 specimens (77.5%) by nCoV-DK; the overall concordance rate was 94.4%: 95.2% for nasopharyngeal swab, 95.5% for saliva, and 85.7% for sputum. The nCoV-DK test effectively detects SARS-CoV-2 in all types of sample including saliva, while reducing the time required for detection, labor, and the risk of human error.

Introduction

Rapid and accurate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for the prevention of outbreaks of coronavirus disease 2019 (COVID-19) in communities and hospitals. The diagnosis of COVID-19 is made by real-time quantitative PCR (RT-qPCR) testing of specimens collected by nasopharyngeal swab (Wang et al., 2020; Zou et al., 2020). However, swab sample collection poses a risk of viral transmission to healthcare workers. Self-collection of saliva reduces the risk to healthcare workers. We and others have shown the efficacy of saliva as a diagnostic tool (Azzi et al., 2020; Iwasaki et al., 2020; To et al., 2020; Williams et al., 2020; Wyllie et al., 2020).

The 2019 Novel Coronavirus Detection Kit (nCoV-DK; Shimadzu Corporation, Kyoto, Japan) eliminates the steps of RNA extraction and purification by using the Ampdirect technology (Nishimura et al., 2010), thus significantly reducing the time required for sample preparation and PCR detection from more than 2 h to about 1 h. In addition, the risk of human error during RNA extraction can be reduced. However, there is a need to elucidate whether saliva samples can be applied to the nCoV-DK, since saliva has high RNase (Pandit et al., 2013). This study was performed to compare the efficacy of the nCoV-DK with direct PCR requiring RNA extraction and purification.

Methods

Samples and PCR

Samples were collected from nine patients with COVID-19, as described previously (Iwasaki et al., 2020). A total of 71 frozen stock samples were available from these patients, with a median of 8 samples (range 2–15 samples) per patient. This study was approved by the institutional ethics board and informed consent was obtained from all patients.

Total RNA was extracted and direct RT-qPCR was performed as described previously (Iwasaki et al., 2020). The nCoV-DK PCR was performed using the corresponding frozen specimens.

Statistical analysis

Agreement between the two methods was assessed using Cohen's kappa. Pearson's correlation coefficient test was performed to identify the relationship of the cycle threshold (Ct)

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Table 1
Comparison of SARS-CoV-2 detection by direct PCR and nCoV-DK method.

		Direct PCR		
nCoV-DK		Positive	Negative	Kappa (95% CI)
Total	Positive	52	3	0.85 (0.70–0.99)
	Negative	1	15	
Swab	Positive	34	2	0.83 (0.60–1)
	Negative	0	6	
Saliva	Positive	13	0	0.90 (0.72–1)
	Negative	1	8	
Sputum	Positive	5	1	0.59 (0–1)
	Negative	0	1	

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; nCoV-DK, 2019 Novel Coronavirus Detection Kit; CI, confidence interval.

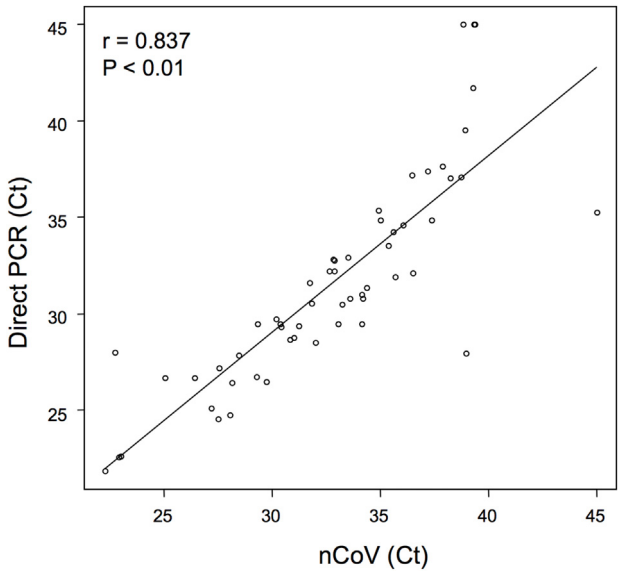

Figure 1. Correlation of Ct values between the direct PCR and nCoV-DK methods. The scatter plot shows the comparison of Ct values between the two methods. Negative samples are those with a Ct of 45, which is the limit of detection.

values between the methods. Statistical analyses were performed with EZR (Jichi Medical University, Saitama, Japan). A p-value of 0.05 was the cut-off for statistical significance.

Results

It was first examined whether the freeze–thaw step could affect the availability of viral RNA for detection. The nCoV-DK PCR was performed on three fresh samples and the corresponding freeze–thaw specimens. Ct values did not change significantly after the freeze–thaw steps.

The viral detection rates were then evaluated in 71 specimens. The virus was detected in 53 (74.6%) fresh samples by the direct PCR and in 55 (77.5%) of the corresponding frozen samples by the nCoV-DK (Table 1). The overall concordance rate of virus detection between the two methods was 94.4% (95% confidence interval (CI) 86.2–98.4%). Inter-rater reliability of the two methods was strong (κ = 0.85), as judged by Cohen's kappa analysis. The concordance rate was 95.2% (95% CI 83.8–99.4%) for nasopharyngeal swab samples, 95.5% (95% CI 77.2–99.9%) for saliva samples, and 85.7% (95% CI 42.1–99.6%) for sputum samples. Figure 1 shows a scatter plot presenting a comparison of Ct values for each sample between the two methods. There was a strong correlation between the two methods (r = 0.837, 95% CI 0.736–0.902, p < 0.01). Significant correlations were also demonstrated for each sample type (swab, r = 0.82, 95% CI 0.673–0.905, p < 0.01; saliva, r = 0.818, 95% CI 0.507–0.94, p < 0.01; sputum, r = 0.945, 95% CI 0.574–0.994, p < 0.01).

Discussion

This study demonstrated that a novel SARS-CoV-2 detection kit – nCoV-DK – is as effective as direct PCR in detecting SARS-CoV-2 in all types of sample. Particularly, it should be noted that saliva is a reliable sample for virus detection by the nCoV-DK even without the process of RNA extraction and purification. There were some discordant results between the two methods. The virus was detected only by direct PCR in one sample, while the virus was detected only by the nCoV-DK in three samples. It is unclear whether these were false-positive or true-positive, since the PCR primers in the two methods are not the same.

In conclusion, the nCoV-DK has advantages over direct PCR, including a shorter detection time by eliminating the steps of RNA extraction and purification, without impairing diagnostic accuracy.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Study design: SI, SF, TT. Data analysis: TF, SI, SF, KH, KS, SO, JS, MN, TT, KT. Sample collection: SN, KK, YY, SK. Writing: TF, SI, SF, JS, TT.

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