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Note

SARS-CoV-2 detection by fluorescence loop-mediated isothermal amplification with and without RNA extraction

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ABSTRACT

Rapid and simple point-of-care detection of SARS-CoV-2 is an urgent need to prevent pandemic. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) can detect SARS-CoV-2 more rapidly than RT-PCR. Saliva is non-invasive specimen suitable for mass-screening, but data comparing utility of nasopharyngeal swab (NPS) and saliva in RT-LAMP test are lacking and it remains unclear whether SARS-CoV-2 could be detected by direct processing of samples without the need for prior RNA extraction saliva. In this study, we compared utility of saliva and NPS samples for the detection of SARS-CoV-2 by a novel RT-fluorescence LAMP (RT-fLAMP). The sensitivity and specificity of the RT-fLAMP with RNA extraction were 97% and 100%, respectively, with equivalent utility of NPS and saliva. However, sensitivity was decreased to 71% and 47% in NPS and saliva samples without RNA extraction, respectively, suggesting that RNA extraction process may be critical for the virus detection by RT-fLAMP.

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Rapid and accurate detection of SARS-CoV-2 is critical for the prevention of outbreaks of coronavirus disease 2019 (COVID-19) in communities and hospitals. Reverse transcription quantitative PCR (RT-PCR) is the standard method to detect SARS-CoV-2 [1]. However, it requires skilled personnel and specialized thermal cycler and takes several hours to obtain results. In contrast, loop-mediated isothermal amplification (LAMP) can generate a large amount of DNA under isothermal conditions within 30 min and allow detection as turbidity or fluorescence [2,3]. Emerging evidences have shown the utility of RT-LAMP for the detection of SARS-CoV-2 [4–8]. RT-LAMP has a potential for direct processing of samples without RNA extraction, which halves turn-around time, but efficacy of SARS-CoV-2 detection by the direct extraction using clinical samples remains unclear [9].

The nasopharyngeal swab (NPS) samples are the standard of the virus detection but self-collected saliva is non-invasive and easy to collect and thus more suitable for mass screening than NSP sampling [6,10–14]. We recently developed a novel RT-fluorescence LAMP (RT-fLAMP) for the detection of SARS-CoV-2. In this study, we evaluated whether RT-fLAMP could efficiently detect SARS-CoV-2 in NPS and saliva samples with or without RNA purification.

We screened 34 viral positive samples (17 NPS and 17 saliva samples) as established by RT-PCR and 27 negative samples (13 NPS and 14 saliva). All the samples had been frozen and thawed before analysis. This study was approved by the Institutional Ethics Board (Hokkaido University Hospital Division of Clinical Research Administration Number: 020–0116), and informed consent was obtained from all patients.

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Following thawing, each sample was divided for RNA extraction and direct extraction. For direct extraction, samples were heated at 95 °C for 10 min to inactivate virus and RNase. Total RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). RT-PCR was performed as previously described [12]. RT-fLAMP was carried out with SARS-CoV-2 RNA Detection Kit Genelyzer KIT (Canon medical systems corporation, Otawara, Japan) using total reaction mixture of 15 μl isothermal mastermix, 4 μl primer mix, 1 μl AMV reverse transcriptase, and 5 μl sample. LAMP amplification and fluorescence detection were performed using Genelyzer FII (Canon medical systems corporation) at 68 °C for 20 min.

The sensitivity and specificity with 95% exact confidence interval (CI) were calculated when the diagnostic results of RT-PCR were considered as the "gold standard". Kendall's coefficient of concordance *W* was evaluated to identify the relation among the cycle threshold (Ct) by RT-PCR and time to positive (Tp) by RT-fLAMP values between the methods. Statistical analyses were performed with R ver 4.0.2. Two-sided significance level was 0.05.

The sensitivity of the RT-fLAMP with RNA extraction were 97% (33/34, 95%CI: 85–100%), 100% (17/17, 95%CI: 80–100%), and 94% (16/17, 95%CI:71–100%) in whole samples, NPS, and saliva samples, respectively (Table 1).

Its specificity was 100% using NPS and saliva samples. Of note, in one patient who showed RT-fLAMP negativity in saliva, Ct value was the highest among patients with RT-PCR positivity (Supplementary Table). There was a significantly strong positive correlation between the Tp value of LAMP test and the Ct value of RT-PCR (Kendall's $W = 0.93$, $P = 0.002$, Fig. 1A). However, the positive rate of RT-fLAMP without RNA extraction against that with RNA extraction decreased to 71% (12/17, 95%CI: 44–90%) and 47% (8/17, 95%CI: 23–72%) in NPS and saliva, respectively (Table 1). The negative samples of RT-fLAMP without RNA extraction tended to have higher Tp (Fig. 1B).

Table 1
Comparison of RT-PCR and RT-fLAMP with or without RNA extraction.

	RT-PCR	RT-fLAMP w RNA ext.		RT-fLAMP w/o RNA ext.	
		Positive	Negative	Positive	Negative
Total (n = 61)	Positive	33	1	20	14
	Negative	0	27	0	27
NPS (n = 30)	Positive	17	0	12	5
	Negative	0	13	0	13
Saliva (n = 31)	Positive	16	1	8	9
	Negative	0	14	0	14

w RNA ext.; with RNA extraction, w/o RNA ext.; without RNA extraction.

A rapid and simple point-of-care detection of SARS-CoV-2 is an urgent need to prevent and control the spread of SARS-CoV-2. In this context, RT-fLAMP has several advantages over the standard RT-PCR: rapid turn-around time, ease of implementation, and potential utility at point of care using simple device. Our results confirm that sensitivity of RT-fLAMP was almost equivalent to that of RT-PCR, as several recent studies demonstrated [4,6,7]. Self-collected saliva is an ideal sample to detect the virus compared to NPS sampling by eliminating uncomfortable process and risk of viral transmission to health care workers. Our results clearly show that saliva is a valuable sample to detect SARS-CoV-2 by RT-fLAMP as an alternative of NPS samples. RT-fLAMP using self-collected saliva is easier and more suitable for mass screening of asymptomatic persons than RT-PCR using NPS by eliminating the need for health care workers and personal protective equipment for sampling, and skilled technicians and specialized thermal cycler.

RT-fLAMP usually requires prior RNA extraction. More rapid detection of the virus is possible if one-step process of RT-fLAMP is feasible without RNA extraction. A recent study suggested the feasibility of the one-step RT-fLAMP without RNA extraction using non-clinical samples [4]. A more recent study demonstrated that sensitivity of the one-step RT-fLAMP was 87% using clinical NPS samples [5]. In our study, sensitivity was 71% using NPS samples but was only 47% using saliva. These results suggest that RNA extraction is critical for better detection of SARS-CoV-2 using saliva. It is possible that high viscosity of saliva may inhibit gene amplification by RT-fLAMP. We recently developed a novel SARS-CoV-2 detection kit for RT-PCR by eliminating RNA extraction and purification [15]. A novel technology to increase sensitivity of RT-fLAMP using saliva without RNA extraction will greatly facilitate rapid point of care detection of SARS-CoV-2.

In conclusion, RT-fluorescence LAMP detects SARS-CoV-2 as effective as PCR. Efficacy of NPS and saliva is equivalent to detect SARS-CoV-2, but RNA extraction process is essential for better detection of SARS-CoV-2 particularly in saliva.

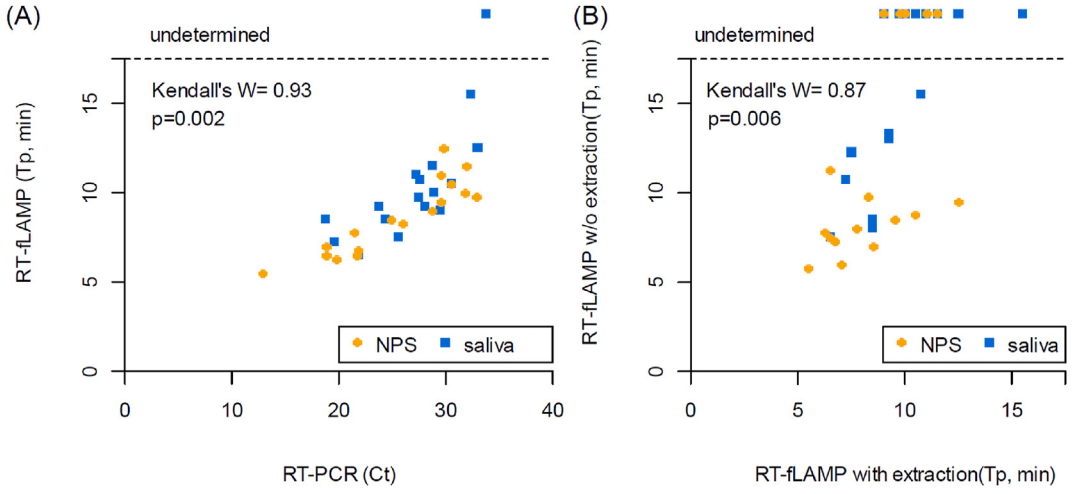

Fig. 1. Correlation of SARS-CoV-2 detection in the RT-PCR and RT-fLAMP methods. (A) A scatter plot shows the relationship between Tp value of RT-fLAMP and Ct value of RT-PCR (n = 34). **(B)** A scatter plot shows Tp value of RT-fLAMP between with and without RNA extraction (n = 33). Kendall's *W* is nonparametric intraclass correlation coefficient. Circles indicates NPS samples and squares indicates saliva samples.

Author contributions

Study design: KT, SI, TF, SF, MT, SN, TT. Data analysis: KT, IY, TF, SI, SF, MT, SN, KH, KS, SO, JS, NM, TT. Sample collection: SK. Writing: KT, IY, TF, SI, SF, NM, JS, TS, TT.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jiac.2020.10.029.

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