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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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Following thawing, each sample was divided for RNA extraction and direct extraction. For direct extraction, samples were heated at 95 ℃ 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 FII (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 ℃ 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).

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) 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.

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.
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.

Acknowledgements

This work was in part supported by grants from Japan Medical Association Research Institute, and Health, Labour and Welfare Policy Research Grants (20HA2002), and Health, Labour and Welfare Science Research Fund (19HA1003).

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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