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Short Communication

Colorimetric detection of SARS-CoV-2 by uracil-DNA glycosylase (UDG) reverse transcription loop-mediated isothermal amplification (RT-LAMP)

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ABSTRACT

Objectives: Preventing reverse transcription loop-mediated isothermal amplification (RT-LAMP) carryover contamination could be solved by adding deoxyuridine triphosphate (dUTP) and uracil-DNA glycosylase (UDG) into the reaction master mix.
Methods: RNA was extracted from nasopharyngeal swab samples by a simple RNA extraction method.
Results: Testing of 77 samples demonstrated 91.2% sensitivity (95% confidence interval [CI]: 78–98.2%) and 100% specificity (95% confidence interval: 92–100%) using UDG RT-LAMP.
Conclusion: This colorimetric UDG RT-LAMP is a simple-to-use, fast, and easy-to-interpret method, which could serve as an alternative for diagnosis of SARS-CoV-2 infection, especially in remote hospitals and laboratories with under-equipped medical facilities.

It has been more than two years since the beginning of the SARS-CoV-2 pandemic. Rapid diagnostic methods such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Notomi et al., 2000) are critically important to minimize the spread of the illness and protect the public.

Because of its high sensitivity criteria, LAMP is prone to carryover contamination. Fortunately, risks of carryover contamination can be reduced by incorporating deoxyuridine triphosphate (dUTP) and thermolabile uracil-DNA glycosylase (UDG) into the master mix (Hsieh et al. 2014). By incorporating dUTP in the LAMP reactions, the amplified products are chemically modified from the target DNA and specifically tagged with uracil. The subsequent LAMP reactions are treated with UDG, making only the natural target DNA intact for amplification and thus decreasing the risk of carryover contamination (Tang et al., 2016). In this study, we included dUTP in RT-LAMP assays and compared its performance with that of dNTP RT-LAMP.

A total of 37 real-time reverse transcription polymerase chain reaction (rRT-PCR)–positive (cycle threshold value ranged from 15.51–38.85) and 40 rRT-PCR–negative clinical swab samples were provided by Hospital Sungai Buloh and Institute for Medical Research, Malaysia. RNA was extracted using a Chelex 100 resin (Bio-Rad Laboratories, Hercules, CA, USA) concentration method as described by Janíková et al. (2021) and Perez et al. (2021), with some modification. The 30% (w/v) Chelex 100 resin was prepared in TE buffer, vortexed vigorously for 10 seconds, and kept at 4°C until further use. Fifteen µL of samples were mixed with 22.5 µL of 30% (w/v) Chelex-TE. The tube was incubated at 98°C for 2 minutes followed by incubation on ice for 2 minutes. The sample was subjected to short spin and the supernatants were further mixed with 1.5 M sodium acetate and 0.3 M sodium hydroxide (NaOH), pH 5.2.

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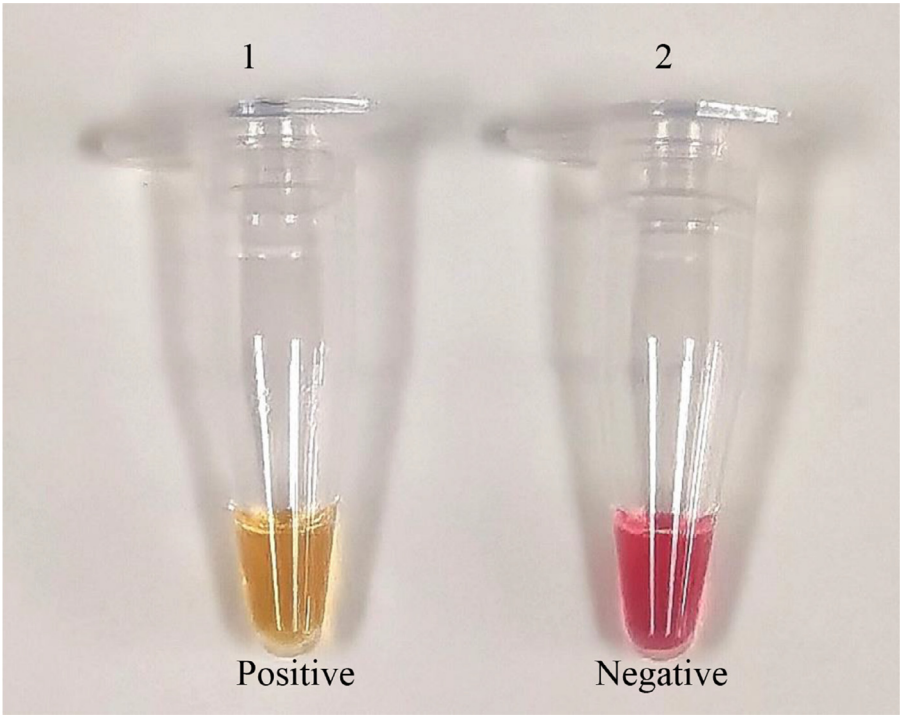

Figure 1. Visualization of the RT-LAMP with the color change of phenol red from pink (negative) to yellow (positive).
Tube 1: positive reaction; Tube 2: negative reaction.
RT-LAMP, reverse transcription loop-mediated isothermal amplification.

Three volumes of ice-cold ethanol were added and vortexed for 5 seconds before being centrifuged for 10 minutes. The tube was air-dried at room temperature for 10 minutes. The RNA pellet was re-suspended with 5 µL of TE buffer and the tube was vortexed for 30 seconds before a quick spin.

UDG RT-LAMP assay was performed in a total of 12.5 μL of reaction mixture and consisted of 1.25 µL of 10 × low-strength buffer (pH 8.3), 0.75 µL of magnesium sulfate, 0.75 µL of *Bst* 2.0 WarmStart DNA Polymerase, 0.15 µL of WarmStart RTx Reverse Transcriptase (New England Biolabs, Ipswich, MA, USA), 1.9 µL of N1 gene primers mix, 4 µL of dNTP/dUTP mix (2 mM deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate and 4 mM dUTP), 0.5 µL of thermolabile UDG, 0.5 µL of RNaseOUT Recombinant Ribonuclease Inhibitor (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of guanidine hydrochloride (GuHCl), 0.2 µL of 10 mM phenol red (MilliporeSigma, St. Louis, MO, United States) and 2 µL of RNA template. Meanwhile, the dNTP LAMP mixture consisted of similar ingredients to those of the UDG RT-LAMP assay except for 3.8 μL of RNase-free water and 0.7 µL of dNTPs.

The N1 gene primers mix used in this study has been reported previously (Lau et al., 2020). The reaction was incubated at room temperature for 5 minutes to allow UDG enzyme to function before the RT-LAMP assay. The amplification was performed in heating block at 65°C for 60 minutes followed by deactivation at 80°C for 2 minutes. Phenol red was added to the master mix for visual detection of the end product by observing color changes. Positive LAMP reaction turned yellow and negative reaction remained in pink (Figure 1). Phenol red is a water-soluble dye that is widely used as a pH indicator in various medical and cell biology tests. At high pH, the dye will show red color, while at low pH, it turns yellow. During the RT-LAMP amplification process, there is production of pyrophosphate and hydrogen ions as byproducts from the incorporation of dNTP/dUTP by the polymerase, which causes pH to drop from alkaline pH to a final acidic pH (Notomi et al. 2000).

Analytical sensitivity was tested using 10-fold serially diluted *in vitro* transcript RNA with known numbers of nucleic acid copies, showing the lowest detection limit as one copy/µL of RNA. Analytical specificity was tested using genomic RNA of coronaviruses (human coronavirus OC43, SARS-CoV, Middle East respiratory syndrome–related coronavirus), adenovirus, human metapneumovirus, influenza A (fluA/H1, fluA/H3A) viruses, influenza B virus, parainfluenza 3, rhinovirus A, respiratory syncytial virus B and enterovirus D68, which demonstrated 100% specificity (Lau et al., 2020). Clinical sensitivity was calculated on the basis of the formula: (number of true positives)/(number of true positives + number of false negatives). Clinical specificity was calculated as (number of true negatives)/(number of true negatives + number of false positives). In this study, the clinical sensitivity and specificity of UDG RT-LAMP were evaluated by using 73 samples. Overall results indicated 91.2% sensitivity (95% confidence interval: 78–98.2%) and 100% specificity (95% confidence interval: 92–100%), respectively.

A commercially available dUTP/dNTP premix was used in this study. There were no false positives of UDG RT-LAMP when compared with dNTP RT-LAMP. Results indicated that six rRT-PCR negative samples were amplified by the dNTP RT-LAMP assay. Meanwhile, UDG RT-LAMP did not amplify all these samples (Supplementary Table 1). We believe that the risk of carryover contamination could be reduced by incorporation of dUTP into the RT-LAMP assay. In addition, our previous study using carryover simulations indicated that UDG can effectively prevent carryover contamination in the LAMP assay up to 0.6 × 10–13 g of LAMP products (Zen et al., 2020). Results revealed that UDG RT-LAMP took a longer time (~9 minutes) for the entire RT-LAMP amplification process compared with normal RT-LAMP with dNTP (Supplementary Table 1). Furthermore, addition of dUTP into the master mix may have slightly affected the sensitivity of UDG RT-LAMP given that three samples were not amplified successfully.

To minimize cost, a novel RNA extraction method from nasopharyngeal swab samples was used in the present study. The commercial kit requires $6.45/reaction compared with our newly developed RNA extraction method, which costs only $2.27/reaction. Therefore, this simple RNA extraction method could be recommended as an alternative for laboratories experiencing shortage of extraction kits.

We managed to develop a simple and cost-effective RNA extraction method from nasopharyngeal swab samples. Coupled with the addition of phenol red as an indicator, this UDG RT-LAMP method is highly recommended for diagnosing SARS-CoV-2 virus infection. Although dNTP RT-LAMP is slightly more sensitive than UDG RT-LAMP, it always has the issue of false positive results due to carryover contamination problems. Our results showed that out of 40 negative rRT-PCRs tested, six samples were amplified by dNTP RT-LAMP. However, UDG RT-LAMP took a longer time for the amplification. The effectiveness of UDG-LAMP can be improved by further optimization of the ratio of dUTP to dNTP in the reaction mix (Kim et al., 2016). As a comparison, rRT-PCR is a gold standard for the diagnosis of SARS-CoV-2; however, it took the longest time to complete the amplification among the three methods (90 minutes). Moreover, rRT-PCR is expensive (~$40/reaction) compared with RT-LAMP ($1.66).

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approvals

Ethical approvals for this study were obtained from the University of Malaya Medical Centre-Medical Research Ethics Committee (202041-8418) and the Medical Research and Ethics Committee of the Ministry of Health of Malaysia (NMRR-20-2344-56994).

Authors' contributions

Meng Yee Lai: study design, data analysis and interpretation, and writing of the original draft; Fatma Diyana Mohd Bukhari: sample testing and writing; Nur Zulaikha Zulkefli: sample testing and writing; Jeyanthi Suppiah: sample collection; Ravindran Thayan: sample collection; Ilyiana Ismail: sample collection; Nur Izati Mustapa: sample collection; Tuan Suhaila Tuan Soh: sample collection; Afifah Haji Hassan: sample collection; Kalaiarasu M Peariasamy: sample collection; Yee Leng Lee: sample collection; Yee Ling Lau: supervision, study design and data interpretation.

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