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Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for point-of-care detection of SARS-CoV-2: a clinical study to evaluate agreement with RT-qPCR

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Abstract

Background The global COVID-19 pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted the need for rapid, accurate, and accessible diagnostics to enable timely treatment and outbreak control. However, current diagnostic tests based on RT-qPCR are insufficient to meet the global testing demand because of their high cost and complexity and supply chain shortages. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a promising alternative to RT-qPCR because of its sensitivity, speed, and robustness to sample inhibitors. Here, we describe the development and optimisation of a sample-to-answer workflow, including a simple lysis and inactivation protocol that provides results in <1h, using inexpensive and readily available reagents. Further, we assess the sensitivity and specificity of the developed RT-LAMP assay against a RT-qPCR.

Methods We collected samples from asymptomatic healthcare workers at The University of Texas MD Anderson Cancer Center and inpatients at Lyndon B Johnson Hospital in Houston, TX, USA. Nasopharyngeal swabs were collected by medical providers and were placed directly into 300 µL of an optimised lysis buffer. Samples were heat inactivated at 95°C for 5 mins before direct amplification in a RT-LAMP assay using previously published primer sets. Heating and real-time monitoring was performed using a Bio-Rad CFX96 thermocycler and an Axxin T8-ISO, a benchtop fluorimeter designed for point-of-care settings. We compared results from the RT-LAMP test with standard-of-care RT-qPCR results on paired nasopharyngeal swabs collected into Universal Viral Transport Media.

Findings The developed RT-LAMP assay demonstrated a limit of detection of 4–5 virions/µL. The test requires a swab, two tubes, prepared lysis buffer, a heat block, pipettes, RT-LAMP reagents, and the real-time fluorimeter. Samples were collected between April 14, 2020 and Aug 12, 2020, and results from 74 enrolled participants were analysed in the optimised workflow. Thirty nine participants tested positive for SARS-CoV-2 and 35 participants tested negative in the hospital-administered RT-qPCR test. All 74 nasopharyngeal swab eluates were tested with our assay on the Bio-Rad CFX96; 72 nasopharyngeal swab eluates were also tested on the Axxin T8-ISO. The developed assay showed sensitivity of 92·31% and 91·89% when tested on the CFX96 and T8, respectively, and specificity of 91·43% and 97·89%, respectively.

Interpretation RT-LAMP could be used for SARS-CoV-2 testing and overcomes the challenges of adapting an assay to a point-of-care instrument. Further, the reduced instrumentation cost and complexity, along with the simple workflow, highlight the potential for implementation of RT-LAMP for SARS-CoV-2 testing in resource-limited settings.

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Declaration of interests
We declare no competing interests.

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