Multiplexed Reverse Transcription Loop-Mediated Isothermal Amplification Coupled with a Nucleic Acid-Based Lateral Flow Dipstick as a Rapid Diagnostic Method to Detect SARS-CoV-2

ABSTRACT

Due to the high reproduction rate of COVID-19, it is important to identify and isolate infected patients at the early stages of infection. The limitations of current diagnostic methods are speed, cost, and accuracy. Furthermore, new viral variants have emerged with higher rates of infectivity and mortality, many with mutations at various primer binding sites, which may evade detection via conventional PCR kits. Therefore, a rapid method that is sensitive, specific, and cost-effective is needed for a point-of-care molecular test. Accordingly, we developed a rapid molecular SARS-CoV-2 detection kit with high specificity and sensitivity, RT-PCR, taking advantage of the loop-mediated isothermal amplification (LAMP) technique. Four sets of six primers were designed based on conserved regions of the SARS-CoV-2 genome: two outer, two inner and two loop primers. Using the optimized protocol, SARS-CoV-2 genes were detected as quickly as 10 min but were most sensitive at 30 min, detecting as little as 100 copies of template DNA. We then coupled the RT-LAMP with a lateral flow dipstick (LFD) for multiplex detection. The LFD could detect two genic amplifications on a single strip, making it suitable for multiplexed detection. The development of a multiplexed RT-LAMP-LFD reaction on crude VTM samples would be suitable for the point-of-care diagnosis of COVID-19 in diagnostic laboratories as well as in private homes.