Title

Reverse transcription loop-mediated isothermal amplification assay for point-of-care molecular diagnosis of COVID-19 in the emergency department

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Abstract

Background and introduction

The COVID-19 pandemic has driven an unprecedent demand for rapid and accurate diagnostic tests for SARS-CoV-2 infection for decision on quarantine, isolation, and treatment. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has several advantages as an emerging point-of-care (POC) diagnostic tool, including amplification at a constant temperature without a thermocycler, simplicity in setup, fast turnaround time, better tolerance of sample impurity and potentially a larger diagnostic capacity.

Objectives

We aimed to evaluate the diagnostic performance of a novel RT-LAMP assay for POC diagnosis of SARS-CoV-2 in the ED using patient nasal swab specimens.

Methods

We developed a novel RT-LAMP assay based on gold nanoparticles (Gold-RT-LAMP, Figure 1) and conducted the assay in the ED fever zone. Consecutive adult ED patients for whom a rapid antigen test (RAT) was clinically indicated and a hospital laboratory reverse transcriptase polymerase chain reaction (RT-PCR) was available as gold standard were recruited between July and October 2022. Diagnostic performance was evaluated in three phases. In phase 1, we conducted the RT-LAMP assay on the leftover RAT buffer solution from the patients. In phase 2, we collected an extra nasal swab and used UltraPure DNase/RNase-free distilled water as the medium to avoid the inhibitory action of the RAT buffer solution. In phase 3, we further optimised the last centrifugation step of Chelex pre-treatment.

Results

In total, we recruited 1,729 patients. In phase 1 (n=971), the sensitivity and specificity of the QIAamp RT-LAMP were 71.4% and 94.0%, respectively. The Chelex RT-LAMP had a sensitivity of 21.4% and specificity of 98.6%. In phase 2 (n=471), the sensitivity and specificity of the Chelex-pre-treated RT-LAMP were 81.8% and 99.7%, respectively. In phase 3 (n=287), the sensitivity and specificity were 98.4% and 100%, respectively. In all the three phases, the RT-LAMP assay outperformed the concurrent RAT in POC diagnosis of SARS-CoV-2. In phase 3, the optimised Chelex Gold-RT-LAMP matched RT-PCR in sensitivity and specificity.

Conclusion

This is the first RT-LAMP study conducted in the ED. This novel assay has the potential of expediting diagnosis of COVID-19 to support clinical decision on in future pandemics.

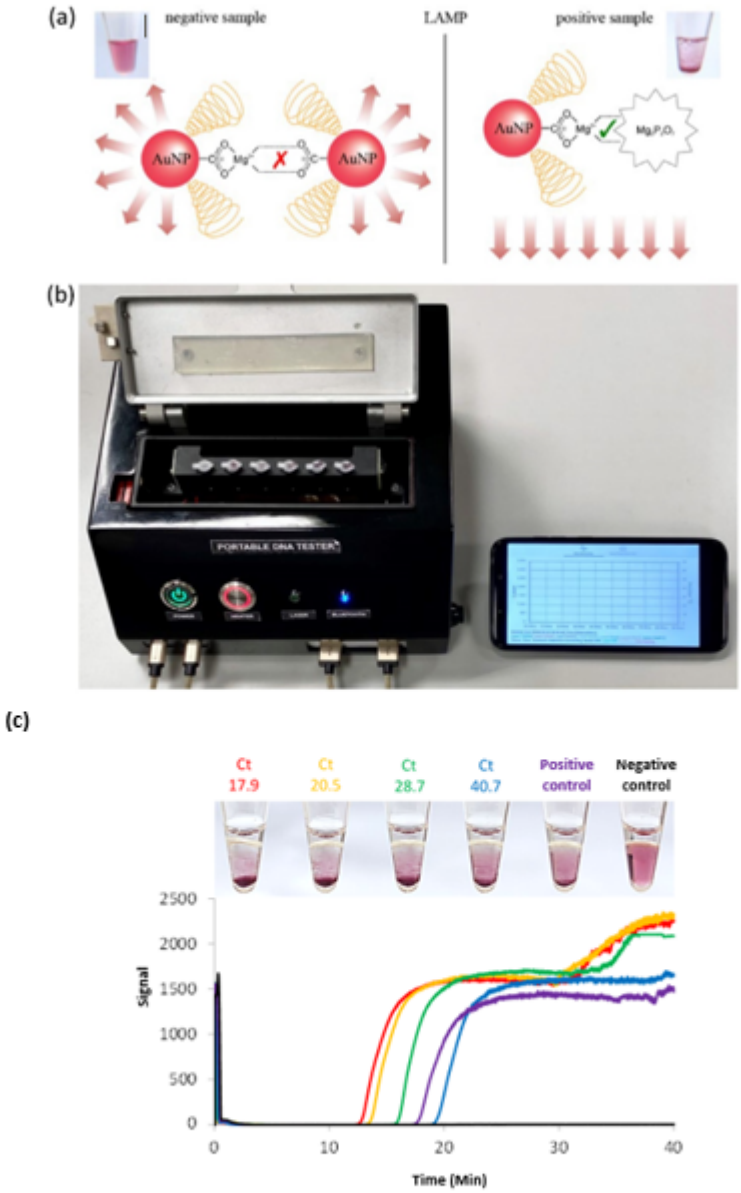

Figure 1. (a) Schematic illustration of the dispersion and precipitation mechanisms of surface-modified gold nanoparticles (AuNPs) in (left) negative (absence of target sequence) and (right) positive (presence of target sequence) loop-mediated isothermal amplification (LAMP) samples. Magnesium pyrophosphate crystals are formed in a positive LAMP sample, thus leading to co-precipitation with AuNPs. (b) An in-house developed low-cost and handheld device for performing the AuNP-based real-time LAMP assay. (c) Plot of device readout signal (520 nm light transmission) against amplification reaction time for different copies of a target sequence.