IVD_01 - Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of SARS-CoV-2 in human samples

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Introduction: Coronavirus disease 2019 (COVID-19) known as severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2), spread rapidly in the world and was declared a pandemic by the World Health Organization (WHO). COVID-19 displays a variety of symptoms, from mild flu to life-threatening conditions. Early detection of an infected person with rapid and sensitive tests is one of the crucial points to control the pandemic. Reverse transcriptase reaction followed by quantitative polymerase chain reaction (RT-qPCR) from nasopharyngeal swabs is the gold standard for molecular diagnosis of SARS-CoV-2. However, the technique has several limitations for application in point-of-care (POC) diagnostics.

Objective: Therefore, the aim of this work was to develop and validate a rapid molecular test based on the reverse transcriptase technique followed by isothermal loop mediated amplification (RT-LAMP) for detection of SARS-CoV-2 in human samples.

Methodology: SARS-CoV-2 strain PE2020-4372 was used in all experiments and RT-LAMP assay conditions were optimized and established. Standardized reaction was used in RT-LAMP test to detect SARS-CoV-2 in a variety of biological samples (nasopharynx, oropharynx, saliva and gargle). Analytical sensitivity assays were performed using serial dilutions of SARS- CoV-2 and *in vitro*-produced transcribed RNA, as well as analytical specificity assays to assess cross-reactivity. Validation of the RT-LAMP assay was performed with 400 clinical samples from patients in the States of Pernambuco and Minas Gerais, Brazil. RT-qPCR was used as gold-standard comparison method. The cost per reaction was calculated based on price of all necessary reagents.

Results: RT-LAMP assay was highly specific for the detection of SARS-CoV-2 in different biological samples in just 15 minutes without needing RNA extraction or using sophisticated equipment. RT-LAMP assay had a high sensitivity, with a detection limit of 8 copies of viral RNA per microliter. Using 400 patient samples, we found similar diagnostic performance when compared RT-qPCR for detection of SARS-CoV-2. As for the cost of each reaction, the value was approximately one real and fifty-five cents (R$ 1.55).

Conclusion: RT-LAMP technology presents itself as a rapid, sensitive, specific and low-cost assay for SARS-CoV-2 diagnosis in different biological samples, including self-collected. Our tool has great potential to produce POC results to assist clinicians and can bring diagnostic decentralization. In addition, we are conducting tests to utilize the RT-LAMP assay in detecting SARS-CoV-2 Variants of Concern (VOCs).

Keywords: Diagnosis; COVID-19; SARS-CoV-2