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DEVELOPMENT OF COLORIMETRIC RT-LAMP FOR RAPID VISUAL DETECTION OF SARS-COV-2 IN CLINICAL SAMPLES

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ABSTRACT

COVID-19 outbreak caused by the novel coronavirus SARS-CoV-2 has forced an urgent need for robust testing strategies to curb the spread of the disease. This study aims to develop, optimize and validate a convenient, yet reliable colorimetric RT-LAMP assay for rapid visual detection of COVID-19. Six newly designed LAMP primers were screened in a singleplex RT-LAMP assay to target on the highly conserve region of nucleocapsid gene (N gene), envelope gene (E gene), open reading frame 1ab (ORF1ab) gene and RNA dependent RNA polymerase (RdRp) gene of SARS-CoV-2, individually. Following optimization, N3 primers presented as the optimal primer based on its amplification performance and colour contrast between negative and positive result. Further study was proceeded with colorimetric RT-LAMP assay using N-3 primers alone. The optimised protocol of the developed assay using N-3 primers represented a limit of detection (LOD) as low as 5 copies of DNA plasmid control in a 12.5 μl reaction within 30 minutes of incubation. However, in real clinical samples, the assay took about 35 minutes of incubation to give yield to positive result. Hence, final optimal reaction condition for N3 primers targeting the N gene was decided at 65ºC for 35 minutes of incubation. In total, the assay took approximately 59 minutes combining 23 minutes of RNA extraction with 35 minutes of isothermal amplification reaction and 1 minutes of direct colorimetric observation. As evidence of high specificity, no cross-reactivity was detected when testing the assay against other non-SARS-CoV-2 viruses, while BLAST analysis proven the low chances of cross-reactivity against most viruses studied. In clinical validation, the developed colorimetric RT-LAMP assay recorded a high degree of sensitivity and specificity at 80.25% and 94.12%, respectively, when tested on 183 purified RNA samples with concordance rate of 87.98% to qRT-PCR assay as the standard reference method. For the conclusion, this assay provides a simple, rapid (less than an hour) yet reliable approach that enables visual detection of SARS-CoV-2 N gene using newly designed primers in molecular diagnosis for a cost-effective COVID-19 prevention and control strategies.

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TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Research Background

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel human coronavirus that was initially discovered in late December 2019 during a mysterious pneumonia outbreak at a seafood wholesale market in Wuhan, China. The emergence of this new virus has taken over the entire globe, posing a serious global public health concern. SARS-CoV-2 is responsible for the ongoing Coronavirus disease 2019 (COVID-19) that was declared as pandemic by the World Health Organization (WHO) in late January 2020 (Chaouch, 2021). COVID-19 patients usually present with non-specific clinical symptoms which are remarkably identical to those of other diseases. However, some patients may not show any symptom at all during infection, making it difficult to diagnose patient infected with the coronavirus (Udugama et al., 2020).

Due to these challenges, molecular detection with various approaches become the favourable method to achieve a more accurate diagnosis of COVID-19 (Lo & Chiu, 2020; Udugama et al., 2020). Among them, quantitative reverse transcription polymerase chain reaction or qRT-PCR has been approved by WHO and US Centers for Disease Control and Prevention (CDC) as the standard assay for detection of the disease due to its performance and reliability (Broughton et al., 2020; Li et al., 2019; Lo & Chiu, 2020). This nucleic acid amplification assay, however, has certain drawbacks that limits its application to meet the current demand as it is a time-consuming procedure that requires the use of high purity samples and sophisticated facilities with well-trained workers to perform the test (Yu et al., 2020). To approach this issue, a quick, simple yet sensitive detection method would be ideal for addressing this matter to allow for rapid diagnosis of the disease especially in point of care setting or in limited resources setting for large scale implementation (Nguyen et al., 2020; Udugama et al., 2020).

Loop-mediated isothermal amplification (LAMP) is a promising alternative molecular diagnostic approach to replace qRT-PCR due to its high efficiency and fast turnaround time (Amaral et al., 2021). The amplification reaction using LAMP