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Fabrication of an inexpensive RT-LAMP thermal cycler for the detection of SARS-CoV-2 VIRUS

Gumel Ahmad Mohammed[1*], *Lawi Isah Abdullahi*[1], *Rahab Gayus*[2]

[1]*Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, 7156 Dutse, Jigawa State, Nigeria*
[2]*Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse, Jigawa State, Nigeria*

***Corresponding Author:**
Prof. Gumel Ahmad Mohammed
Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, 7156 Dutse, Jigawa State, Nigeria
Email: *am.gumel@fud.edu.ng*
Phone: *+2348161101920*

ABSTRACT

The rapid detection of SARS-CoV-2, the virus responsible for COVID-19, is critical for managing the ongoing pandemic. The research paper addresses the limitations of the current diagnostic standard, real-time reverse transcription-polymerase chain reaction (RT-PCR), which, despite its sensitivity, is hindered by lengthy operation time, requiring high cost of commercially available thermal cyclers which limits its widespread application, particularly in resource-limited settings. In response, the study proposes the development of a thermal incubator for a novel, rapid, and cost-effective colorimetric method based on Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP). The RT-LAMP approach requires minimal equipment and can be effectively deployed in remote areas without reliable electricity, allowing for the rapid screening of 96 samples in under 20 minutes. The method was observed to outperform conventional RT-PCR in terms of accessibility and operational efficiency. The study aims to contribute significantly to the containment of COVID-19 spread in Nigeria and beyond. Ultimately, this research aspires to provide a practical solution to enhance diagnostic capabilities in resource-limited settings.

Keywords:*COVID-19, SARS-CoV-2, PCR, Virus, Isothermal amplification, PCR, RT-LAMP*

INTRODUCTION

The coronavirus disease of 2019 (COVID-19) is a global pandemic caused by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Since its emergence in Wuhan province of China in late 2019, the disease has been a major global public health challenge with third world nations experiencing the worst scenario. COVID-19 is highly contagious and to date, thus if not properly managed it can lead to fatality. The disease can manifest its symptoms (fever, headache, dry cough, excessive sneezing, Shortness of breath or difficulty breathing, muscle aches, sore throats, conjunctivitis etc.) within two to fourteen days post exposure(WHO, 2020). The disease spread through air presenting infection by contamination with aerosols or droplets as infected person speaks, thus necessitating the wearing of facial mask and social distancing strategy in public.

COVID-19 is known to be highly contagious and if not properly managed it can lead to fatality. From 30 December through 11 October, over 37 million COVID-19 cases and 1 million deaths have been reported globally (WHO, 2020). Nearly half of these cases (48%) and 55% of the mortality were reported in the region of the Americas with the USA, Brazil and Argentina accounting for the greatest numbers of new cases and deaths in the region (WHO, 2020). In Nigeria, from 27 February to 26 October 2020, a total of 61992 COVID-19 cases were confirmed, 3397 active cases and reported 1130 mortality(Akande et al., 2021). Of the confirmed COVID-19 cases in Nigeria, 66% were reported to be asymptomatic at diagnosis (Ayodele et al., 2021).

Several nations have employed combinations of containment and mitigation strategies(WHO, 2021); in fact, early diagnosis of COVID-19 is known to be vital in effective management of the disease and controlling the viral spread within the population. On this note, employing simple and rapid tests for detecting existing COVID-19 infections are critical in managing widespread of the disease. The current molecular diagnosis of COVID-19 is based on the detection of SARS-CoV-2 nucleic acid using real-time reverse transcription-polymerase chain reaction (RT-PCR)(Tahamtan & Ardebili, 2020). The steps involve nucleic acid extraction from the specimens, gene amplification, and detection. Although RT-PCR is sensitive, unfortunately, it suffers major setbacks. It is technical extensive and requires expensive equipment. Similarly, the method takes longer processing time of about 4-8 h. These setbacks have been great obstacles on effective deployment of such diagnostic tests especially in developing countries or in remote areas where there is no enough medical facilities.

RT-LAMP exhibits simplicity, increased sensitivity, specificity, significantly rapid, and does not require expensive reagents or equipment(Thompson & Lei, 2020). The method was previously employed in molecular diagnosis of several infectious diseases spanning from tuberculosis, to SARS, MERS etc. The method only requires simple equipment such as controlled temperature incubator, bench-top mini centrifuges, heat blocks, and micropipettes. The develop RT-LAMP method will be so simple that it can be deployed at the remotest region of Nigeria and operate in absence of electric power. The turn-around time of the develop method would be short that 96 samples can be screened in less than 20 minutes with high degree of accuracy.

In this paper, we proposed to overcome the ELISA and RT-PCR limitations by fabricating a thermal incubator which will be used to develop a rapid and inexpensive colometric method based on nucleic acid amplification by Reverse Transcription Loop mediated isothermal Amplification (RT-LAMP) for mass on-site screening of COVID-19 infection. We hypothesized that the robustness and rapidity of the newly developed RT-LAMP assay for screening SARS-CoV-2 RNA would surpass that of conventional expensive and difficult to deploy RT-PCR assay. The cost effectiveness, specificity, rapidity and accuracy of the proposed method could be the next 'Holy Grail' in containing the speedy spread of COVID-19 among Nigeria population and the world at large.

Materials and Methods

A total of 53 leftover nasopharyngeal swab samples in Becton Dicson universal viral transport medium (BD, USA), from both symptomatic and asymptomatic individuals attending for testing facilities in Dutse, Jigawa State,Nigeria tested for SARS-CoV-2 by RT-PCR were used to validate the described RT-LAMP assay. Comparisons were based on RT-PCR positive samples, and RT-PCR negative samples.

Sample preparation

Samples were thawed and briefly spun down in a mini-centrifuge to collect cells and debris. An aliquot (50 µl) was drawn from the bottom and transferred to labeled Eppendorf tube. Serial 10X dilutions of samples were prepared in nuclease-free water (Thermo Fisher, USA).

RT-LAMP assay and controls

RT-LAMP was carried out using the RT-LAMP Colorimetric Kit (RayBiotech, USA) and a reported LAMP primer set (Table 1) targeting the N gene of SARS-CoV-2 (Schellenberg et al., 2021). Tenfold mixture of the six primers in the set was prepared by first suspending all primers separately in nuclease-free water at a concentration of 100 µM. Thereafter, 16 µl each of FIP/BIP, 4 µl each of LF/LB, 2 µl each of F3/B3, were combined with 56 µl nuclease-free water to make a final volume of 100 µl. Reactions were set up in a final volume of 20 µl (10 µl 2X Master Mix, 2 µl 10X primer mix, 6 µl nuclease-free water and 2 µl sample, either diluted or undiluted) and was incubated in the fabricated thermal cycler at 65^{o}C for 30 min. Samples were then removed and placed in an ice box for a few minutes to sharpen colour contrast, and visually assessed. Bright yellow colour indicates a positive result, while magenta indicates a negative result. Tests with orange or pink colour were considered failed reactions and re-tested.

Table 1 RT-LAMP primers used in the research

Primer	Sequence
FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
Loop F	GGACTGAGATCTTTCATTTTACCGT
Loop B	ACTGAGGGAGCCTTGAATACA
F3	TGGCTACTACCGAAGAGCT
B3	TGCAGCATTGTTAGCAGGAT

Results

Design of thermal cycler

The schematic design of the thermal cycler is illustrated in Fig. 1. The thermal cycler comprised of three parts i.e. thermal balancing blocks, thermal micro controller and thermal heat sink. The thermal balancing blocks are made of high thermally conductive aluminium to achieve uniform temperature around each sample tube.

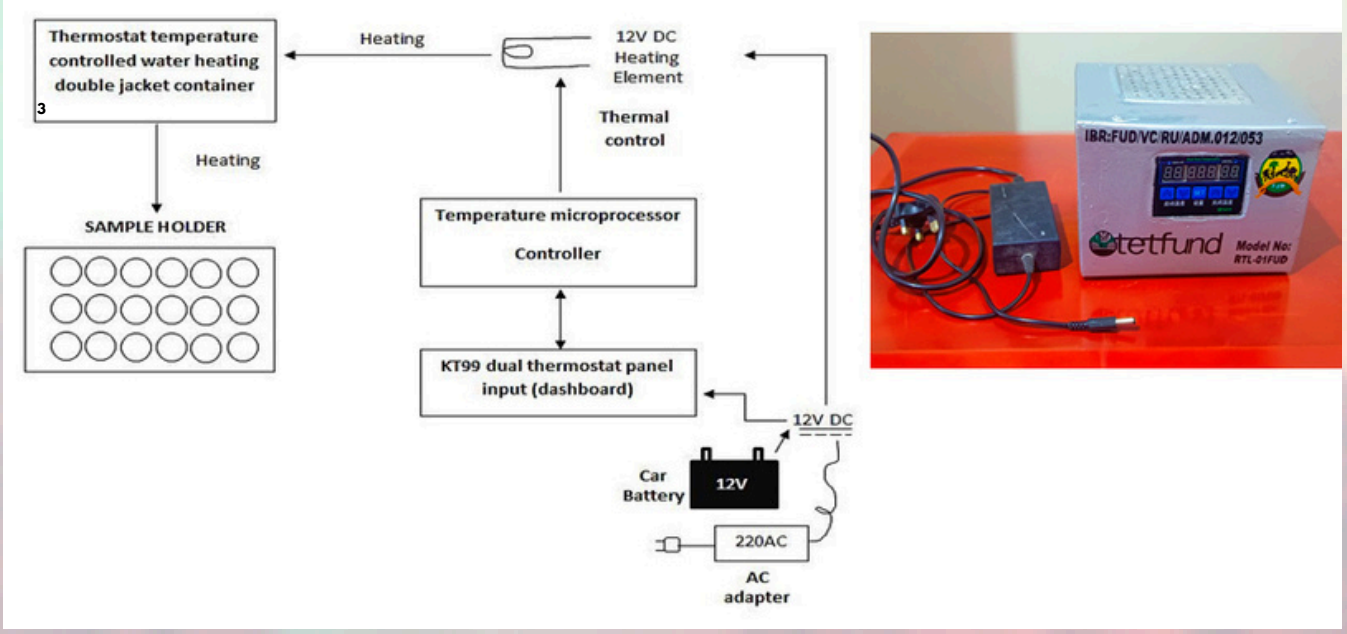

Figure 1: Schematic design of the fabricated RT-LAMP thermal cycler

Sensitivity and Interpretation of colour change

Sensitivity of the RT-LAMP assay was tested using heat-inactivated sample was 80% compared to RT-PCR of purified RNA extracts. Positive samples were observed to be bright yellow after 30 min incubation. In some instances, the sample color transitioned from magenta to pink to orange, but did not progress to yellow during the 30 min incubation period. The color change observed in this sample is indicative of a failed reaction or a genuine negative result. (Fig. 2).

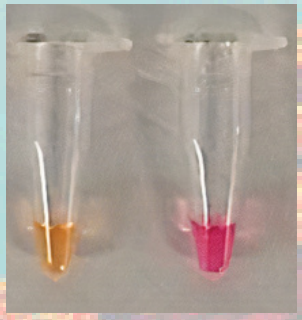

Figure 2: Chromogenic Product of RT-LAMP after 30 min of incubation. Yellow colour indicates positive reaction and magenta indicates negative.

Discussion

This study confirms that one of the fastest, simplest, most cost-effective, and scalable procedures for detecting SARS-CoV-2 nucleic acid is currently reliable only for strong positives, although it exhibits great specificity compared to RT-PCR detection. Other studies have demonstrated comparable low sensitivity but good specificity (Schellenberg et al., 2021; Sherrill-Mix et al., 2021). An initial investigation indicated that RT-LAMP had more sensitivity than RT-PCR in identifying the virus in an asymptomatic carrier observed over many days (Thompson & Lei, 2020). Similarly to the current investigation, sensitivity was greatest in strong positives, indicating that only those with high viral loads may reliably be detected. Recent work has demonstrated that SARS-CoV-2 could not be cultivated from samples with Ct values more than 24 and/or longer than 8 days post symptom onset (Schellenberg et al., 2021). This observation indicates that the RT-LAMP assay proposed here would detect all of those most likely to have culturable (and consequently infectious) virus, as has been shown when the two have been compared directly(Pu et al., 2022). Many methods have been introduced or adapted to quick detection of SARS-CoV-2 at the point of care(Thompson & Lei, 2020). Despite limits in reality, all tests used on a bigger scale, with correct messaging regarding the limited relevance of negative results, could be highly valuable if strong positives are detected and acted upon more rapidly . Several jurisdictions employ the Panbio Ag Rapid Test Device to identify positives early, presuming that negative results should be confirmed (Albert et al., 2021; Linares et al., 2020; Torres et al., 2021). One recent study demonstrated that none of those who were test discordant (positive by RT-PCR but negative using Panbio) had culturable virus in their sample (Boujemaa et al., 2023), indicating potentially reduced transmissibility by those characterized as “false negatives” in existing techniques. Other investigations have demonstrated that the sensitivity of the Panbio devices drops among persons with no symptoms (Ngo Nsoga et al., 2021) or when utilizing self-collected items such as nose swabs and saliva. The influence of mutations on our capacity to reliably detect SARS-CoV-2 with primers based on older genome sequences is an area of significant concern, necessitating ongoing vigilance and updates of primers being utilized. Mutations across the genome (including in the N gene targeted in this work) are regularly reported around the world (Holland et al., 2022; Marhoon & AL-Obaidi, 2022; Wang et al., 2020). Other ways to decrease the likelihood of false negative RT-LAMP tests due to mutations, for example, by targeting more than one gene (typical in RT-PCR assays), have also recently been published (Zhang et al., 2021). Molecular diagnostic approaches that use raw or little-processed samples are fastest, but less sensitive, since the background chemistry of a raw sample may contribute uncontrolled variability. Potential inhibitors or other impurities may impair the reliability of both positive and negative outcomes.

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Conflict of Interest

The authors declared no existence of conflicting interest.

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