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A Portable Device for LAMP Based Detection of Sars-Cov-2

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Abstract: This paper reports the design, development, and testing of a novel, yet simple and low-cost portable device for the rapid detection of SARS-CoV-2. The device performs loop mediated isothermal amplification (LAMP) and provides visually distinguishable images of the fluorescence emitted from the samples. The device utilises an aluminium block embedded with a cartridge heater for isothermal heating of the sample and a single-board computer and camera for fluorescence detection. The device demonstrates promising results within 20 minutes using clinically relevant starting concentrations of the synthetic template. Time-to-signal data for this device are considerably lower compared to standard qPCR machine (~10-20 minutes vs >38 minutes) for 1×10^5 starting template copy number. The device in its fully optimized and characterized state can potentially be used as simple to operate, rapid, sensitive, and inexpensive platform for population screening as well as point-of-need SARS-CoV-2 detection and patient management.

Keywords: SARS-COV-2; Loop-mediated isothermal amplification; Portable device.

1. Introduction

The ongoing severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) pandemic has so far, as of 18 July 2021, caused around 190 million confirmed cases of infection and more than 4 million deaths worldwide.[1] Since its first identification as the cause underlying an outbreak of unidentified pneumonia cases in Wuhan city, central China, the disease has rapidly spread to all inhabitable continents. Though many vaccines have been proved to be effective against the pandemic, the importance of non-pharmaceutical interventions such as physical distancing, contact tracing, and quarantine are still effectively used to control the spread of the disease. [2]However, experts have emphasized that social distancing measures cannot be effective without extensive testing for SARS-CoV-2. Around 80% of infected persons show mild or no symptoms at all, thus majority of the infections go undocumented. Mathematical modelling of spatiotemporal dynamics of SARS-CoV-2 spread have shown that undocumented cases are the source of infection for a large majority (79%) of documented cases, thus emphasizing the need for mass testing.[3] Experiences from South Korea,, ,Germany, Singapore, Taiwan and Hong Kong suggest that it is possible to stem the onslaught of pandemic through surveillance and strict quarantine measures, aided by widespread testing .[4]

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is the current gold standard diagnostic test for SARS-CoV-2. Although the qRT-PCR method is well tested and assays for newly identified organisms can be developed within a very short period of time, it has several drawbacks. The test is relatively expensive and can only be carried out in dedicated centralized diagnostic laboratories. The test itself takes 4-6 hours to complete, however as the samples need to be shipped to distant laboratories, actual

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turnaround time may be more than 24 hours.[4] In a rapidly spreading pandemic, particularly in the context of resource limited regions, reliance on expensive centralized testing may prove to be a bottleneck in implementation of control measures and clinical decision making. Therefore, there is an urgent need to develop point-of-care (POC) testing devices. Earlier this year, the WHO expert group identified the development of rapid POC tests for SARS-CoV-2 as the first of eight research priorities .[5]

Single temperature nucleic acid amplification methods such as loop-mediated isothermal amplification (LAMP) have emerged as simple alternatives to qRT-PCR. The LAMP method is highly specific, takes less than an hour to complete, is less affected by non-target templates and well-known PCR inhibitors present in biological samples, can be implemented even without DNA extraction, and lastly can directly amplify RNA templates without cDNA conversion[6] and complex sample handling procedures.[7] Fluorescence based real time monitoring of LAMP reaction is by far considered most sensitive compared to other methods. [6] However, detection usually relies on the fluorescence sensors integrated in conventional benchtop thermocyclers. In recent years, efforts have been made to develop instruments with dedicated fluorescence readout for isothermal amplification (e.g., Genie® II by OptiGene). However, these instruments are still relatively expensive because of the complex design. A simple, inexpensive, robust, and portable fluorescence detection enabled LAMP device can immensely strengthen the battle against SARS-CoV-2. Such a field deployable device will obviate the need for sophisticated sample storage and shipment chain thus reducing the testing cost and time gap between sample collection and delivery of results. Using LAMP for an RNA virus like SARS-CoV-2 can further reduce the cost and analysis time by eliminating the need for RNA isolation and cDNA conversion steps. Taken together, these features make a LAMP based portable/handheld device an excellent candidate for mass screening and point-of-care patient management in our time of widespread pandemic. Herein we report a novel, low cost and portable device capable of performing LAMP reaction and sensitive monitoring of fluorescence in real time. Using this device, we demonstrate the proof of concept for detection of clinically relevant concentrations of SARS-CoV-2 specific sequences.

2. Materials and Methods

2.1 Design and experimental conditions for SARS-CoV-2 specific LAMP assay

A 204 bp long synthetic target corresponding to the RNA dependent RNA polymerase (RdRp) region of SARS-CoV-2 genome (Australian isolate GenBank: MT050414.1) was used as template in all the LAMP reactions. Template and primer sequences are given in Table 1. Target sequence was isothermally amplified using WarmStart® LAMP Kit (DNA & RNA), (Cat # E1700S, NEB Australia) in a 25 µL reaction as per manufacturer's instruction. The reaction mix consisted of 12.5 µL of WarmStart LAMP 2X Master Mix, 0.5 µL of 50X fluorescent dye, and 2.5 µL of 10X primer mix (final concentrations: FIP/BIP 1.6 µM, F3/B3 0.2 µM). 1 µL of synthetic target of known copy number was used and the reaction was made up to 25 µL using nuclease-free distilled water (Cat # 10977015, ThermoFisher Australia).

2.2 Device design

The device consists of two subsections, the thermal control subsystem and the fluorescence monitoring subsystem. The thermal control subsystem is responsible to maintain the temperature required for loop-mediated isothermal amplification. A custom-designed aluminum block (40×30×10 mm^3) with a cartridge heater (MG-1007, Makergear) embedded in it acts as the heater platform for sample holding. A custom-designed through-hole was made in the aluminum block so that the conventional PCR tube with the sample can be tightly inserted into it. The design of the sample placing hole is in such a way that there

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is an efficient heat transfer between the aluminum block and sample. The through-hole

Table 1: Sequences of templates and primers

Name	Sequence
F3	GTGTGCTCAAGTATTGAGTGAA
B3	CTGTGTTGTAAATTGCGGACA
FIP	TTGTGG-CATCTCCTGATGAGGTTCTTTTATGGTCATGTGTGGCGGTT
BIP	GTCAAGCTGTCACGGCCAATGTTTTTCGGCAATTTT-GTTACCATCAGTAG
Synthetic target	GTGTGCTCAAGTATTGAG-TGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAAC-CAGGTG-GAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATA GTGTTTTTAACATTTGTCAAGCTGTCAC-GGCCAATGTTAATGCACTTTTATCTACTGATGG-TAACAAAATTGCCGATAAGTATGTCCGCAATTTACAACA CAG

also helps for efficient fluorescence capturing on successful LAMP of the sample. An LM-35 temperature sensor was fixed to the aluminum block using Stars 922 heat conductive adhesive. The temperature of the aluminum block was fed back to a custom developed Atmega-328p microcontroller board (Arduino) programmed with a PID temperature controller algorithm. The power to the cartridge heater was controlled by the microcontroller board through a solid-state relay (KSJ30D100-L, Kudom, Jaycar, Australia) to regulate the temperature of the aluminium block to the set point temperature (65^0 C).

Fluorescence monitoring subsystem consists of a raspberry pi B+ single-board computer connected with a 5MP Camera (B0032, Arducam), 7-inch touchscreen monitor (CE04459, Core electronics) and a blue light source. The blue light source was custom built by circularly arranging 30 blue LEDs (ZD0180, Jaycar,Australia). A graphical user interface (GUI) was developed using Python and installed in the single board computer to control the blue light source, camera and the monitor. The camera and the blue light source were arranged vertically and directed opposite to each other with the sample holder placed between them. The light from the blue light passes through the sample in the plastic vial and the fluorescent light emitted from the sample is received by the camera placed beneath the sample holder. The fluorescent light emitted by the sample was filtered using a green optical filter before being received by the camera to improve the signal to noise ratio. The user can either record a video for 30 minutes or take snapshots of the illuminated sample at intervals of 10 minutes o for 30 minutes using the GUI. The python code was programmed to turn on the blue light in 10 minutes interval to avoid the photo bleaching of the fluorescent dye. A mini hot air blower was arranged to blow hot air at $100\,^0$C to the top side of the PCR tube to prevent the condensation of the sample and improve the efficiency of fluorescent excitation. The schematic of the experimental setup is depicted in Figure 2a.

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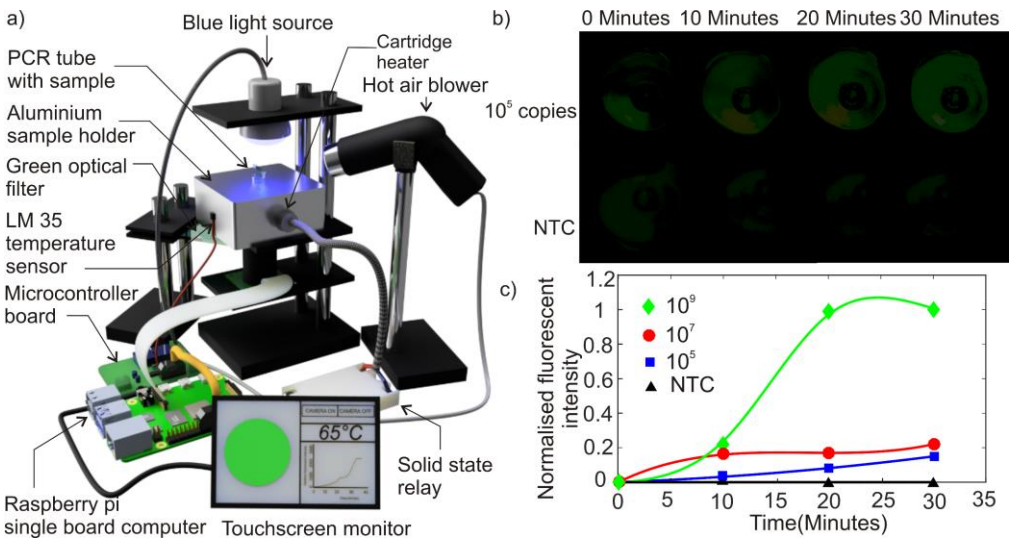

Figure 2. a) Schematic of the experimental setup. b) Photographs of fluorescence emitted from samples upon LAMP. c) Variation of normalized fluorescent intensities of various samples with respect to time

Figure 1. a) The LAMP fluorescence signal (RFU) *vs*. incubation amplification curve graph. b) Corresponding melt peaks of the above reactions. c) Agarose gel (2%) analysis of the LAMP amplified products. Left to right; 50bp DNA ladder, NTC, $1 \times 10^3 - 1 \times 10^9$ starting C. Ns.

2.3 *Experimental*

PCR tube with 25-µL sample was loaded into the sample holder heated to 65ºC. Image capture of illuminated samples was initiated by pressing 'Snapshots' button in GUI. Images were captured at 0, 10, 20 and 30 minutes of the LAMP reaction and were subsequently saved on the single board computer for data analysis and visual inspection. Each of these images were converted into RGB format and the sum of green component of each pixel of the image was automatically calculated by the python code. This sum of green component of all the pixels of the image was considered as the intensity of fluorescence of corresponding image. The fluorescent intensity value thus obtained for the image taken at 0 minute was considered as the background noise and was subsequently subtracted from the fluorescent intensity values obtained for images taken at 10, 20, and 30th minute for offset correction. The offset corrected values of fluorescent intensities were automatically plotted by the algorithm

To compare the efficiency of our device, real time monitoring of change in fluorescence levels in LAMP reactions was carried out in parallel on CFX96 Touch Real-Time PCR Detection System - Bio-Rad. Samples were incubated on 65°C for 33 minutes and fluorescence signal was recorded in FAM channel after every minute. The reaction was stopped by denaturing *Bst* 2.0 and RTx enzymes at 85°C for 5 minutes. The melt curve analysis of amplified products was carried out by heating for 5 seconds each between 65°C and 95°C at 0.5°C increments. Data collection was enabled at each increment of the temperature. Four different starting template copy numbers (C. Ns), 1×10^3, 1×10^5, 1×10^7 and 1×10^9, were tested. A no template control (NTC) reaction was also included. The reaction

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products were subsequently analyzed on a 2% agarose gel in TAE buffer stained with SYBR™ Safe DNA Gel Stain (Cat # S33102, Thermo Fisher Australia) with 50bp DNA ladder (Cat. # 10416014 Invitrogen™) as size marker.

3. Results and discussion

3.1 SARS-CoV-2 specific LAMP assay using conventional thermocycler

Four different starting template copy numbers (C. Ns), 1×10^3, 1×10^5, 1×10^7 and 1×10^9, were tested. A no template control (NTC) reaction was also included. As shown in Figure 1a, only 1×10^7 and 1×10^9 concentrations showed significant increase in fluorescence at the end of 33 minutes reaction, while no amplification was observed in the two lower concentrations as well as NTC. Melt curve analysis also showed sharp peaks at 82°C and 83°C only for 1×10^9 and 1×10^7 C. Ns, respectively (Figure 1b). Similarly, no amplified DNA product could be visualized for 1×10^5 and 1×10^3 starting copy numbers on agarose gel (Figure 1c). We also tested longer incubation times for lower concentrations. The fluorescence signal significantly higher than NTC (> 5-folds) was observed in 1×10^5 concentration only after incubations longer than 38 minutes while for 1×10^3 starting C. N, no significant difference was found even after 45 minutes incubation (data not shown). Moreover, incubation times longer than 40 minutes lead to primer dimer formation in NTC wells thus increasing the chances of false positive interpretation of fluorescence-based data.

3.2 Performance of portable device for LAMP based detection of SARS-CoV-2

For device-based LAMP reaction, three different initial template C. Ns 1×10^5, 1×10^7, 1×10^9 as well as NTC were used. There was a clear increase in fluorescent signal intensity with respect to time for all the positive controls while there was no significant visible increase in fluorescent intensity for the NTC. The fluorescent intensity change was distinguishable by the naked eye by looking at the photographs taken. Images of the fluorescence emitted from the sample after every 10 minutes interval for sample with 1×10^5 initial template copy number and with NTC is depicted in Figure 2b. The images were processed with ImageJ software to quantitatively evaluate the variation in fluorescent intensity with respect to time. The numerical equivalent values of the fluorescence were offset corrected and normalized using the equation.[8,9]

$$I_s^* = (I_s - I_{s0})/I_{max} \quad (1)$$

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Where I_s is the fluorescent intensity of a sample measured at a given cycle, I_{s0} is the fluorescent intensity of that sample at the beginning of thermal cycling and I_{max} is the maximum fluorescent intensity recorded among all the samples in the experiment. The variation of normalised fluorescent intensity of various samples with respect to time is shown in Figure 2c. Viral load in patients even at prodromal stage has been estimated to be in the range of 1×10^5 copies per oro- or nasopharyngeal swab specimen.[10] Therefore, this was considered an appropriate benchmark for minimum detectable copy number using our device. Our device was able to detect visible fluorescence signals in concentrations as low as 1×10^5 within 10-20 minutes (Figure 2b) which is considerably shorter than the standard qPCR machine (>30 minutes). The experimental setup shown in figure 2.a is subsequently organized into a portable device for user friendly operation. The only difference between the experimental setup and the portable device model is that a copper block embedded with cartridge heater replaces the hot air blower to avoid condensation of the sample on the top side of the plastic vial. The detailed exploded view and appearance of the portable device is depicted in figure 3a. Figure 3b illustrates the physical appearance of the device. Figure 3 c is the screenshot of the GUI and the LAMP results obtained for sample with $1x10^5$ initial copy numbers after 30 minutes of the reaction.

4. Conclusions

We have demonstrated the applicability of a proof-of-concept device for LAMP based detection of clinically relevant concentrations of SARS-CoV-2 within 10 to 20 minutes. The device proposed in this study utilizes a raspberry single-board computer, Atmega 328 microcontroller (Arduino), Raspberry pi camera, and other simple basic electronic com-

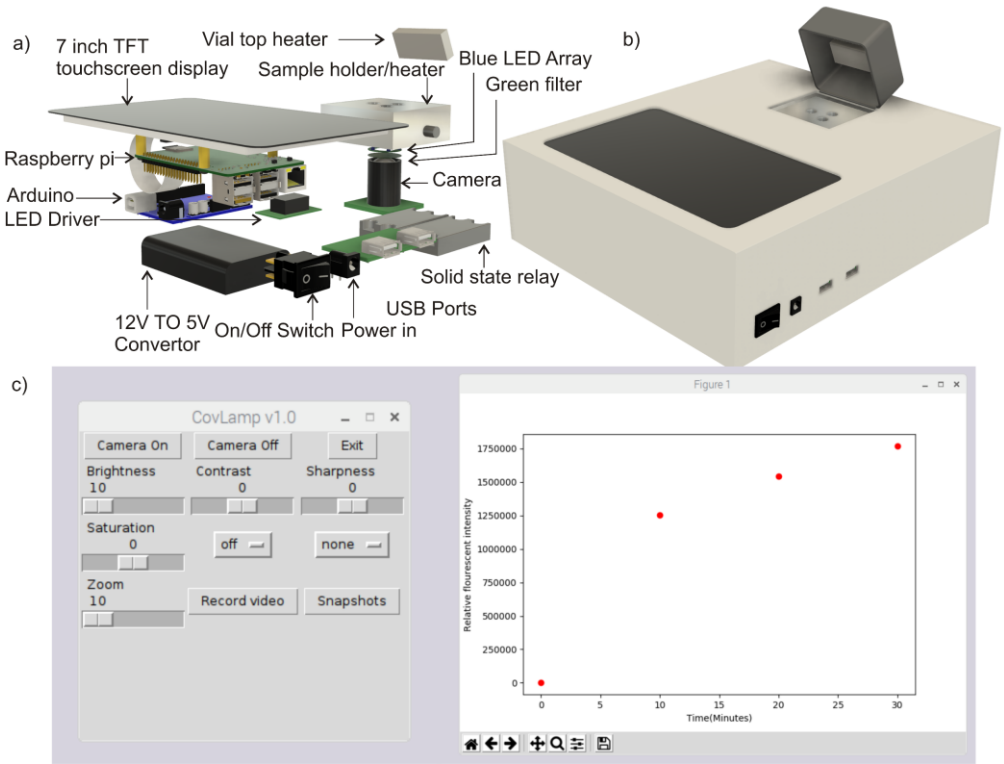

Figure 3. a) Exploded view of the portable device. b) External physical appearance of the portabl device. c) Screenshot of the graphical user interface and the result of the image analysis for sample with 10^5 initial copynumbers.

ponents in its design. The total cost of the components of proposed device may be below A$ 150. The cost can be further reduced to below A$ 100 by using Raspberry pi zero single-

board computers and further optimization of the design. One other salient feature of the proposed device is that it can be easily assembled by anyone with a fundamental level of expertise in electronics using locally available components thus potentially helping to circumvent the mass scale production and supply issues. The proposed handheld device can be easily deployed in places such as airports or hospital emergencies where rapid detection and subsequent quarantine is highly recommended. Its application in POC settings will not only facilitate in rapid clinical management decisions, but will also reduce the burden of centralized testing laboratories. The device opens an avenue for high throughput, low cost, and rapid detection of SARS-CoV-2 in the present pandemic scenario. Moreover, the proposed handheld LAMP device can be easily adapted for the LAMP based rapid detection of many other diseases. Furthermore, droplet-based microfluidics would allow for faster two-phase flow heat transfer,[11] droplet manipulation[12] and more effective LAMP reaction. In the near future, flexible systems allow for the integration temperature sensing and heating on low-cost substrate such as paper,[13,14] the concept described in this paper can be further simplified to lower the assay cost.

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Conflicts of Interest: There are no conflicts to declare.

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