SALIVA-BASED SARS-COV-2 SELF-TESTING WITH RT-LAMP IN A MOBILE DEVICE (SLIDE)

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ABSTRACT

We developed a fully integrated SARS-CoV-2 nucleic acid testing (NAT) device using a self-collected saliva sample. This platform consists of a ready-to-use reagents cartridge, an easy-to-use smartphone interface, and an ultra-compact and less-expensive analyzer. Our system automatically handled the complexity of heat-inactivated sample preparation, pressure-driven sample dispensing, real-time RT-LAMP reaction and detection, and data processing, storage, and upload. With a turnaround time of ~45 minutes, our reverse transcription loop-mediated isothermal amplification (RT-LAMP) achieved the limit of detection (LoD) of 5 virus particles/μl in the saliva sample. We believe our self-testing platform will have an ongoing benefit for COVID-19 control and fighting future pandemics.

KEYWORDS

SARS-CoV-2; POCT; Self-testing; RT-LAMP

INTRODUCTION

Coronavirus disease 2019 (COVID-19) became a worldwide pandemic in early 2020[1], and it was rapidly announced as a public health emergency of international concern by the World Health Organization (WHO)[2, 3]. As of March 2022, there are more than 400 million confirmed cases, and 6 million deaths of SARS-CoV-2 reported globally[3]. A lot of effort has been made into vaccine development, and it has been distributed in communities[4]. However, due to the fast mutation nature of the RNA virus and so many asymptomatic cases, all countries still face an unmet need to achieve a rapid, sensitive and reliable way to tackle the global and urgent problem. To reopen the communities and recover the economy, implementing routine level screening of healthy individuals should be a solution to minimize the risk of spreading. So far, nucleic acid amplification test (NAAT), such as RT-PCR, is the gold-standard technique due to its high sensitivity and specificity [5-8]. However, laboratory-based NAAT requires highly trained personnel, dedicated facilities, and instrumentations. Typically, results can be done within 2 hours, but many counties usually delay up to 7 days due to lack of facilities. Postponing obtaining molecular testing results will increase the virus spread. To alleviate these bottlenecks, we developed a fully integrated SARS-CoV-2 nucleic acid testing (NAT) device. SLIDE platform consists of a ready-to-use reagents cartridge, an easy-to-use smartphone interface, and an ultra-compact and less-expensive analyzer. It automatically handled the complexity of heat-inactivated sample preparation, pressure-driven sample dispensing, and real-time RT-LAMP reaction and detection, and data processing, storage, and upload. With a turnaround time of ~45 minutes, we achieved a limit of detection (LoD) of 5 virion/μl of a saliva sample. We believe our SLIDE self-testing platform will have an ongoing benefit for COVID-19 control and fighting future pandemics.

RESULTS & DISCUSSION

Overall Design and Module Validations

Overall Design. The overall design of the SLIDE analyzer is shown in **Figure 1a**. It consists of five seamlessly integrated modules controlled by a microcontroller unit (MCU): an optical module for fluorescence excitation and detection, two thermal modules, a piezo micro pump module, a connectivity module, and a power module. **Figure 1 b** shows a photograph of the assembled SLIDE analyzer and the smartphone interface.

Optical Module. The optical module consists of three independent excitation and detection units. Each unit has a LED excitation source (λ=470 nm) and a CMOS color sensor for real-time fluorescence monitoring. The excitation and the detection were arranged to be perpendicular to each other to minimize the excitation interference on the fluorescence signal。 To characterize the quantification ability of the optical module, we tested different calcein concentrations from 0 to 25 μM and measured the fluorescence intensity for 10 minutes. **Figure 1c** showed the mean and standard deviation of the relative fluorescence unit (RFU) as a function of the calcein concentration. A linear fit with R^2= 0.98 confirmed the quantitative capability of the optical module.

Micro pump Module. The sample dispensing and mixing is accomplished on the cartridge using a micro piezo pump. The volumetric rate of the micro piezo pump is controlled by the frequency and the driving voltage (fixed at 140V in our case). To characterize the micro pump, we tested the volumetric rate at different frequencies. As expected, the volumetric rate increased linearly with the operation frequency (R^2= 0.99, **Figure 1d**). This relationship provides us with the capability to modulate the liquid flow rate on the cartridge through programming the operation frequency.

Power Module. A rechargeable 1300 mAh Lithium polymer battery (14.43 Wh) was used to power our analyzer. To estimate the power consumption for each run, we used a power meter to characterize the voltage, current, and power during a complete cycle of the test. **Figure 1e** shows a 20 minutes segment of the measurement result. Before reaching the target temperature, the heaters continuously work at a high current (1.7 A for 95 °C and 2.2 A for 65 °C). After reaching the target temperature, the heater starts to change states between on and off to maintain the temperature. The total energy consumed is 3.02 Wh in each 45 minutes test, and we can perform four tests before recharging.

Thermal Module. We designed two independent thermal modules. One is for heat- inactivating the saliva and performing the thermal lysis at 95°C. The other is for controlling the temperature of the RT-LAMP reaction at 65°C. **Figure 1f** shows the temperature profile of the 95 °C thermal modules. The heating block reached 95 °C after 2.05 min of operation, while the saliva in the cartridge took 5.02 min. This delay is due to the non-ideal thermal coupling and the different specific heat capacity between the heating block and the cartridge. Nevertheless, the saliva can be sufficiently lysed at 95°C for 5 min within 10 mins from a cold start. For the heating module controlling the RT-LAMP reaction, we observed that the mean and the standard deviation of the temperature in the master mix solution is 64°C and 0.38°C, respectively (**Figure 2g)**.

Connectivity Module. A smartphone app was developed to assist the user in conducting the test. The SLDIE analyzer and the smartphone communicated through Bluetooth LE protocol. The app could provide test instructions, acquire data, and make positive and negative calls for easy interpretation of the test results. The app could also save the test results into a spreadsheet, which could be

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saved on the local smartphone or uploaded to cloud-based storage (Google Drive).

Automated Saliva Processing on The Cartridge

The cartridge was fabricated from polymethyl methacrylate (PMMA) using a laser cutting machine. It consists of three layers: top-loading layer, middle microchannel layer, and bottom covering layer (**Figure 2a),** and all pieces were bonded layer by layer using an adhesive solvent. It features a saliva collection chamber (250 µl) for loading and heat-inactivate the collected saliva, three trapping chambers for metering 10 µl of a processed saliva sample, and three reaction chambers with preloaded RT-LAMP master mix (30 µl), and a waste chamber for collecting the remaining saliva sample. Microchannels connect all these chambers, and the flow through the channels is controlled by micro pump and valves.

Over the past decade, numerous elegant designs have been demonstrated to control the liquid transfer in microfluidic systems using different types of valves. The wax valve has become popular in microfluidic due to its straightforward mechanism. The operation of a wax valve is based on the principle that the paraffin wax goes through a phase change from solid to liquid or liquid to solid as the wax temperature increases and decreases. Since our system involves the thermal module for the assay reaction, the wax valve naturally became the most straightforward choice to convert thermal energy into mechanical motion for flow control. Two paraffin wax valves are involved in each unit. Paraffin wax valve 1 locates between the trapping chambers and the reaction chambers. It separates the trapping chamber and the reaction chamber to block the flow during the sample trapping process to ensure only 10 µl of sample is transferred. Paraffin wax valve 2 locates in the middle of the releasing channel, which connects to atmospheric pressure. There are two purposes for this design. 1) Paraffin wax valve 2 serves as a liquid resistance to ensure no single unit can directly connect to the atmospheric pressure. This is a really important step to maintain consistency and repeatability among the three units. 2) It also prevents the amplicons from escaping to the environment to cause contamination issues.

Here, we use one unit as an example to illustrate working mechanisms (**Figure 2b).** First, the Saliva sample is heat-inactivated at 95°C for 5 minutes in the collection chamber. Then the saliva is pushed along the microchannel to the waste chamber, which is connected to atmospheric pressure. Along these microchannels, three 10 µl heat-inactivated saliva is taken by the trapping chambers for the following step reaction**.** Since the microfluidic channel of the PMMA layer is cut by a laser and the channel walls are hydrophobic, the trapping chamber can be filled without bubbles. The average trapping volume is 10.25 ±0.27 µl. The difference between the three chambers was less than 2.5%. Next, the heating block temperature was increased to 65 °C to open the paraffin wax valves 1 and 2. Part of the saliva will automatically flow into the reaction chamber by gravity. The rest of the saliva will be pushed by the following 30 pressure pulses at 1Hz with 10% duty at 5.6 ml/min. These pulses can help the saliva sample well mix with the RT-LAMP master mix. **Figure 2c** shows one example of the automatic sample dispensing processes.

Self-testing workflow

The overall workflow from sample collection to analysis using our SLIDE device is shown **Figure 3.** This test needs a ready-to-use reagents cartridge, a 3D printed portable ultra-compact analyzer, and an easy-to-use smartphone interface. First, the saliva sample is acquired from the patient. With the help of the saliva collection aids, the patient would self-collect ~120 µL of clean saliva into a cartridge. Next, the user can tighten the screw cap to completely seal the cartridge and then connect the cartridge with the piezo pump through Luer-lock compatible inlet port. After inserting the cartridge into the analyzer, users need to connect a smartphone to the SLIDE analyzer through Bluetooth and initiate the test. This process takes < 2 min hands-on time and is the only manual testing step. Next, the SLIDE analyzer automatically handles other complexities, including heat-inactivated sample preparation, the pressure-driven sample dispensing, real-time RT-LAMP reaction and detection, and data processing, storage, and upload. Briefly, the virus in the saliva sample is inactivated and lysed at 95°C for 5 minutes. Next, the 10 µl processed saliva will be automatically transferred into the reaction chamber and mixed with a preloaded RT-LAMP master mix by a fined-tuned sequence and parameter combination of microfluid design, piezo pump pressure, wax valve. The reaction will then be initiated on the device and held at 65 °C for 30 min for LAMP amplification. In the reaction, SARS-CoV-2 RNA is transcribed into DNA and amplified to billion copies of amplicons, producing fluorescent signals for detection. The LED excitation module shines through the cartridge during the amplification, and the fluorescence is detected by photodiodes secured perpendicular to the LED excitation module. The real-time amplification curve is displayed on the phone screen. Two possible decisions will be made based on the build-in voting algorithm: If more than or equal to 2 assays shows a sharp RUF increase, it is identified as positive. Otherwise, it is determined as negative. At the end of the test, users can upload their personal information, raw data, and results to the cloud. The whole process takes about 45 min with no user intervention.

Performance Evaluation with Mock Saliva Sample

After validating all the subsystems, we set out the experiment to test the performance of the SLIDE instrument for the automatic process of the sample preparation, amplification, detection, and result analysis. Here, we used a previously validated SARS-CoV-2 RT-LAMP primer set[9] against the highly conserved N region with a modified fluorescent concentration of SYTO9 (**Figure 4a).** We formed mock SARS-CoV-2 positive samples by spiking the healthy saliva sample with different concentrations of heat-inactivated SARS-CoV-2 virus particles. The final concentration of the mock sample ranges from 1 to 10^4 virion/µl.

Firstly, we manually validated the sample preparation and RT-LAMP reaction throughout the whole process. We heat-inactivated all the spiked saliva samples in the heating block for 5 min at 95°C. Next, 10 µl of the inactivated sample was taken by pipette and transferred into the 30 µl of the RT-LAMP master mix. After mixing the reagents with a vortex mixer, the reactions were performed using a benchtop PCR machine. Triplicate mock samples were performed at each concentration. 3/3 samples with the concertation higher than 5 virion/µl showed RFU values sharp increase, while no sample with 1 virion/µl showed sharp changes in RFU. The LoD is about 5 virion/µl (50 virion/reaction), which is on par with our previous RT-LAMP assay validation with purified RNA sample [9].

Same sample sets were performed, and similar results were obtained using the SLIDE device with the automatic process. To further evaluate the analytical sensitivity of the test, we tested more samples at low concentrations from 1 to 10 virion/µl using a SLIDE analyzer. 9/9 samples with the concertation of 10 virion/µl showed positive. 8/9 samples with 5 virion/µl and 3/9 with 5 virion/µl showed positive, respectively. The threshold to classify an amplification curve as positive or negative was 50 RFU. These validate this automatic process does not negatively affect the RNA quality and quantity for downstream detection. To estimate the LoD of the test, we fitted a logistic curve for the hit rates at different virion concentrations. The hit rate is defined as the number of

amplified samples over all tested samples. The LoD is estimated to be 6.5 virion/ µl (65 virion/reaction) at the 95% confidence level. This LoD is comparable with the LoD(6–12 copies/µL) using FDA-proved qRT-PCR assays with the same heat-inactivated saliva sample preparation method[10]. **Figure 4b** shows the inversely proportional relationship between the time to positive and virus particle concentration. As expected, the standard deviation among the times to positive increases as the virus particle concentration decreases in both manual and automatic results. The time to positive in the SLIDE instrument seems slightly slower than in the PCR machine. Due to the similar LoD in the two methods, the RNA quality and quantity should be the same after sample preparation. Therefore, we think this delay came from the different thermal coupling efficiency in these two systems and dynamic range differences in the optical detectors.

Saliva Clinical Sample

Two clinical saliva samples (one known positive and one known negative) were obtained through an approved institutional review board (IRB). All the samples were coded to remove information associated with patient identifiers. The RT-PCR performed the initial diagnosis as the reference method to benchmark our SLIDE. The amplification curves (raw data) are shown in **Figure 4b c&d.** The threshold to classify an amplification curve as positive or negative was set at 50 RFU based on the NTC sample. In 30 minutes, all of the chambers in the positive sample showed sharp RFU increases and stabilized at the value above the threshold, while no obvious RFU changes were observed in the negative sample. Based on the voting system for result making: more than or equal to 2 out of three amplified assay is identified as positive, otherwise is determined as negative. The pre-identified positive and negative samples by RT-PCR were also determined as positive and negative by our SLIDE device.

CONCLUSION

This paper demonstrates a fully integrated system for rapid (<45 min) self-testing for the SARS-CoV-2 virus from saliva samples. This fully portable approach can detect the virus rapidly without needing an RNA extraction kit and pipetting steps. All other complexities are handled automatically by the SLIDE analyzer, including heat-inactivated sample preparation, the pressure-driven sample dispensing and mixing real-time RT-LAMP reaction and detection, and data processing, storage, and upload. Our automatic system shows a great quantitative agreement with the manual process using a benchtop PCR machine (outside the cartridge). The analytical sensitivity (Limit of detection) against SARS-CoV-2 virus particle spiked saliva sample is 5 virion/µl. With these two clinical saliva samples, our device shows 100% agreement with the RT-PCR method. The promising results of the present study could likely be extended for use with saliva samples for noninvasive, portable, rapid, and scalable self-testing for COVID-19. Considering the limited reagent lifetime at room temperature, we will address the challenge of storing and transporting the liquid phase on the microfluidic reagent with reagent lyophilization in the future study.

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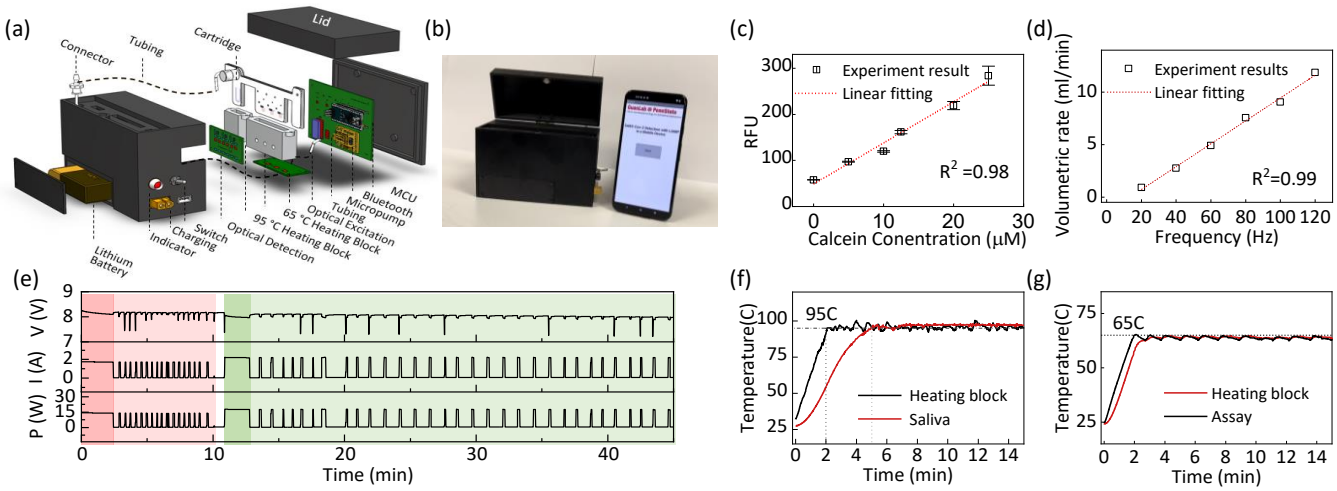

Figure 1*: SLIDE Instrument design and validation. (A) Schematic of the SLIDE device showing components in an exploded view. (B) Photograph of the SLIDE analyzer and the smartphone interface. (C) Characterization of the optical sensor (D) The temperature profile of the heating block and the liquid (saliva/assay) for 95°C virus heat inactivation and 65°C RT-LAMP reaction. (E) Characterization of the piezo pump frequency with the flowrate*

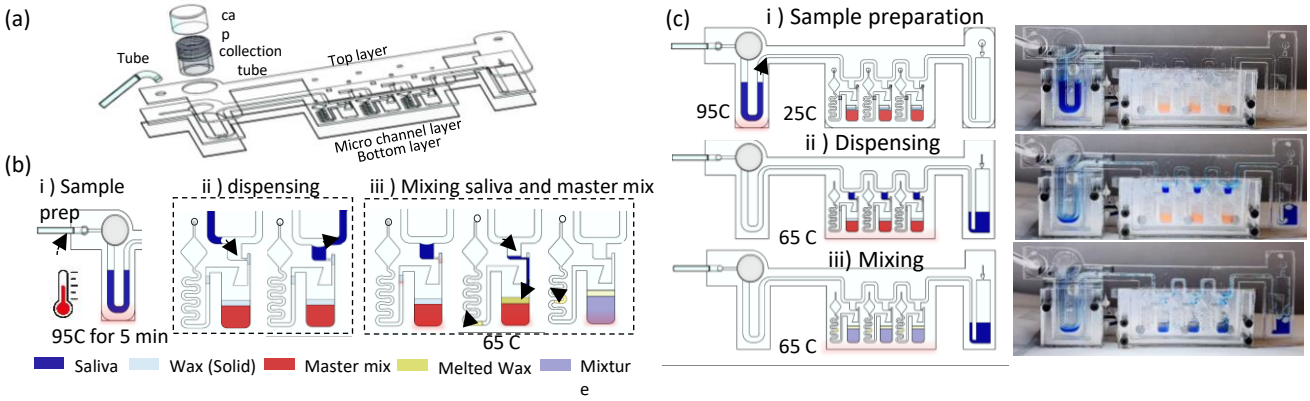

Figure 2. Illustration for the automated sample preparation dispensing and mixing on cartridges enabled by micropump.

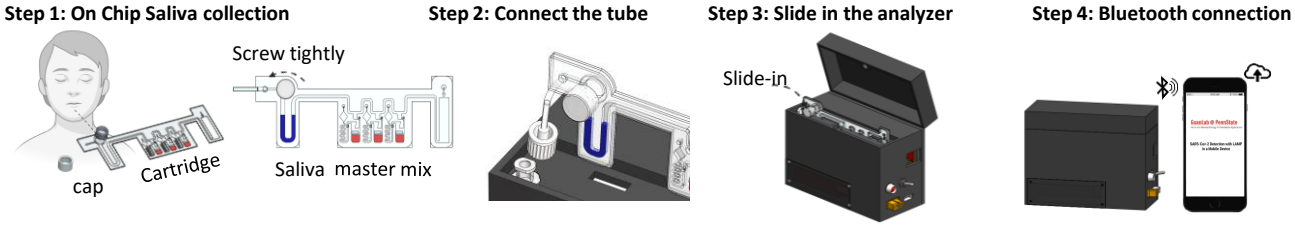

Figure 3. Overall operation workflow of SARS-CoV-2 SLIDE device. . The user self-collects 120 μL of saliva into the cartridge tube. After collection, the sealed cartridge is then inserted into the analyzer . The analyzer is connected to a smart phone through Bluetooth.

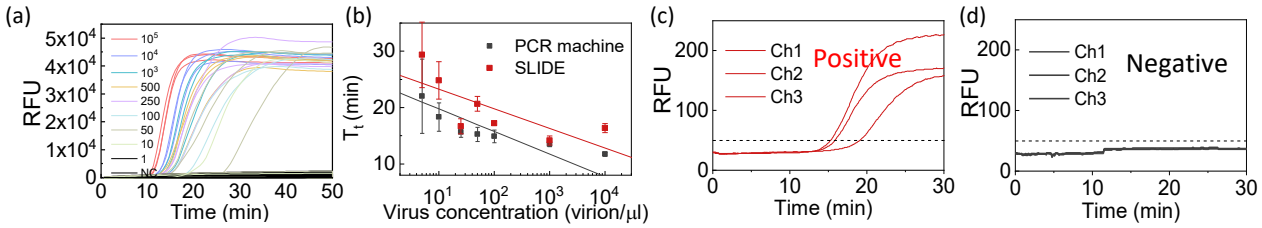

Figure 4: (a) RT-LAMP assay validation. (b) The inversely proportional relationship between the time to positive and virus particle concentration showed in both methods (PCR machine and SLIDE device. (c-d) Validation of the SLIDE automation system using saliva clinical samples.