SLIDE: Saliva-based SARS-CoV-2 Self-Testing with RT-LAMP In a Mobile Device

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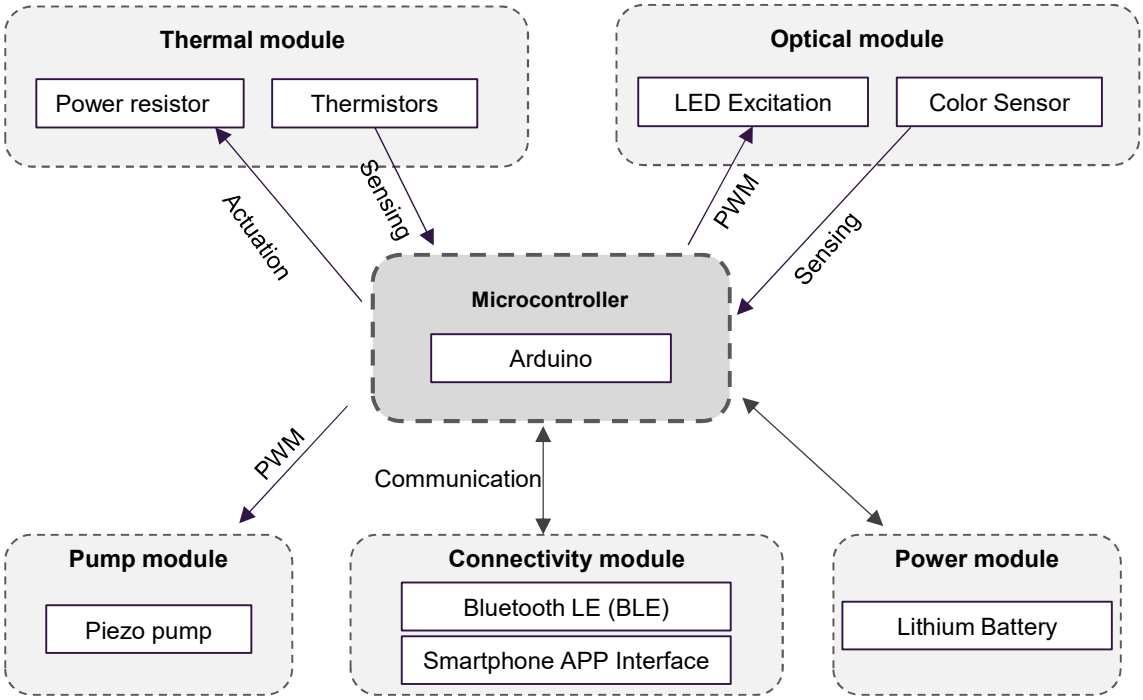


Figure S1. The system diagram of the SLIDE analyzer. One Arduino nano microcontroller incorporates five subsystems: thermal module, optical module, pump module, connectivity module, and power module.

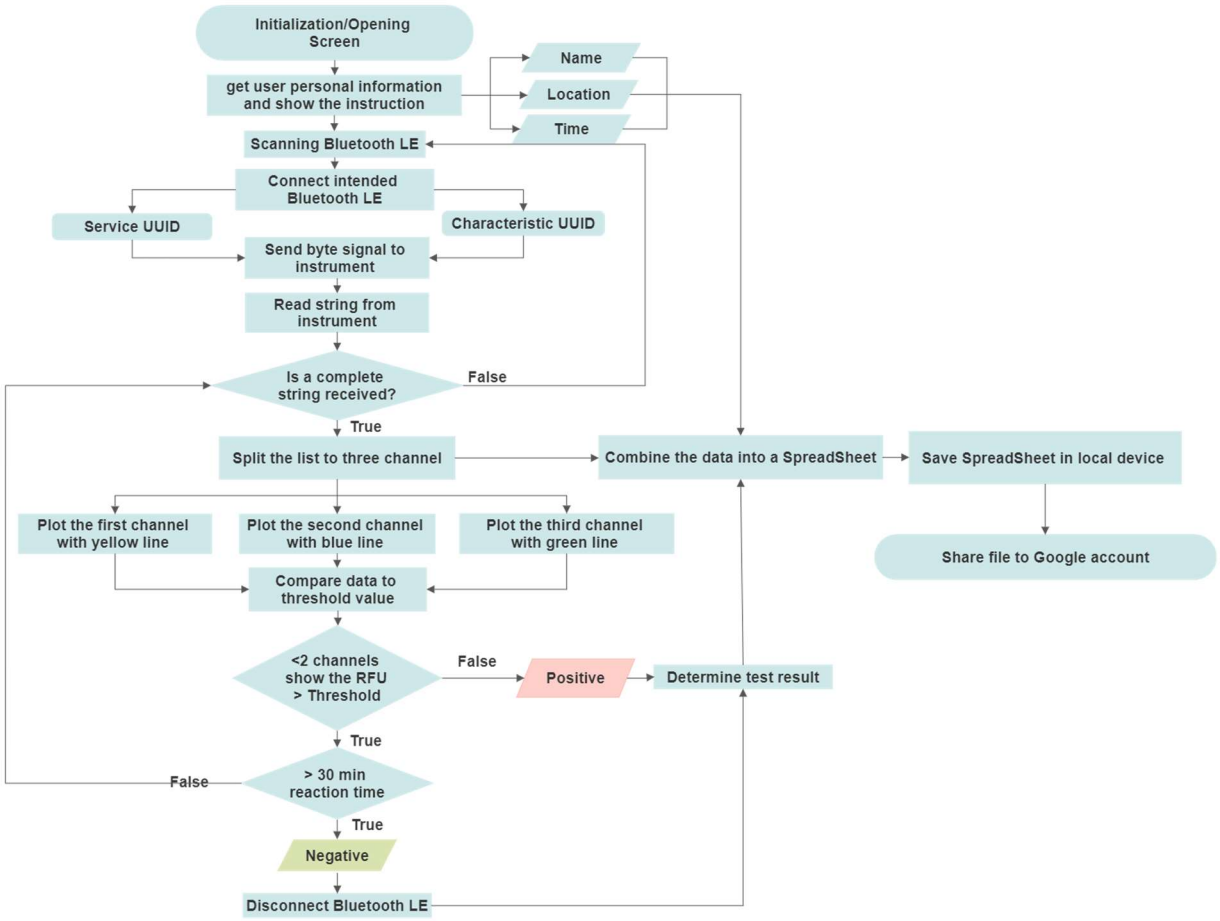


Figure S2. The flow chart of the android App development. First, the App interface guides users in providing their personal information. Second, we set up Bluetooth communication. App interface scans and connects the Bluetooth LE around the analyzer. The communication protocol can be built using the Service UUID and Characteristic UUID of the Bluetooth LE, enabling the data communication function between these two devices. The third part is the real-time data transfer and plotting. We added two check bits at the beginning and the end of the string to ensure accuracy. After confirming the check bit of the received string from the analyzer, the string value will be split into three channels and plotted with different colors. Meanwhile, the split data in each channel is compared with the threshold value (RFU 50) to make the decision. If more than or equal to two channels have three successive data greater than the threshold, the test result will be identified as a positive. Otherwise, the App will continue to receive the string value from the analyzer. If no positive result has been determined after 30 minutes of the amplification, the test result will be negative. The APP will combine personal information, color sensor data in each channel, and test results into a spreadsheet. This file can be saved on the local device and uploaded to a Google drive.

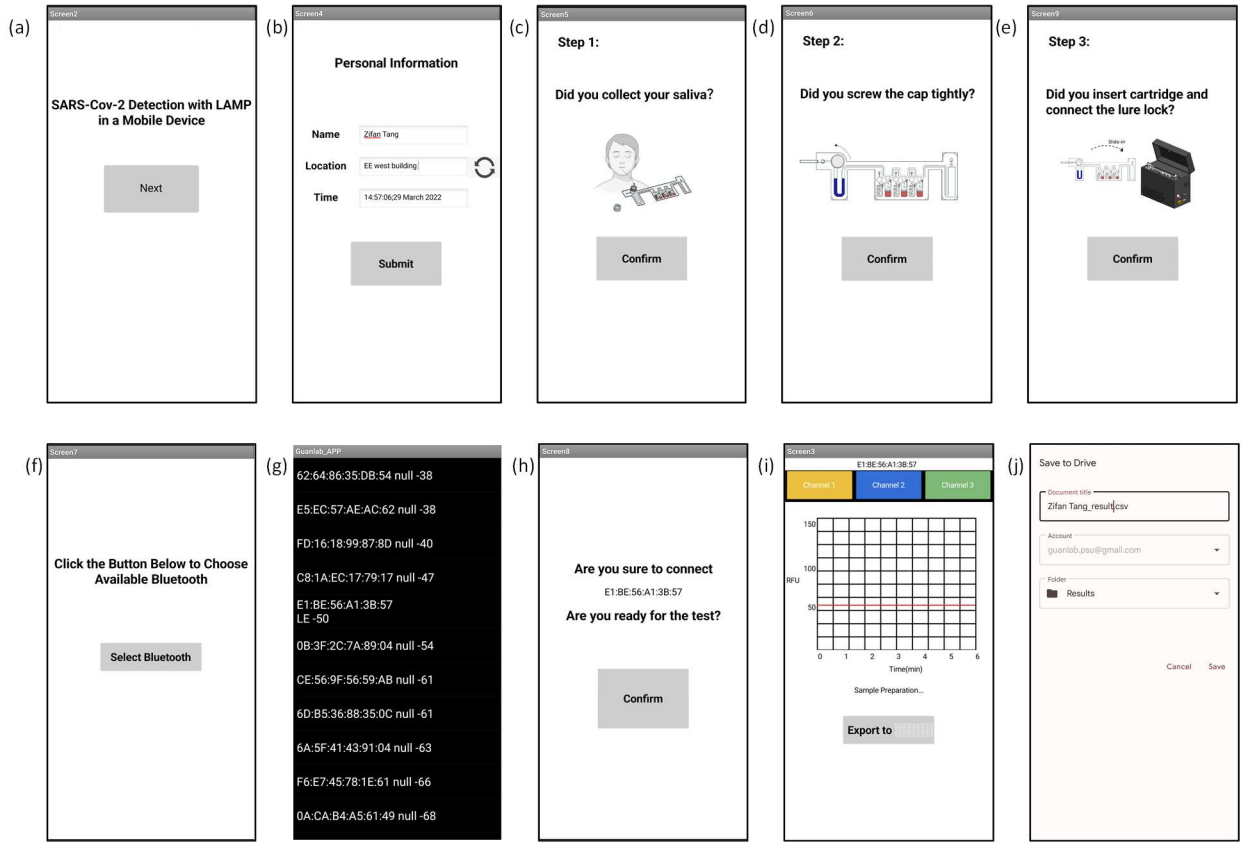
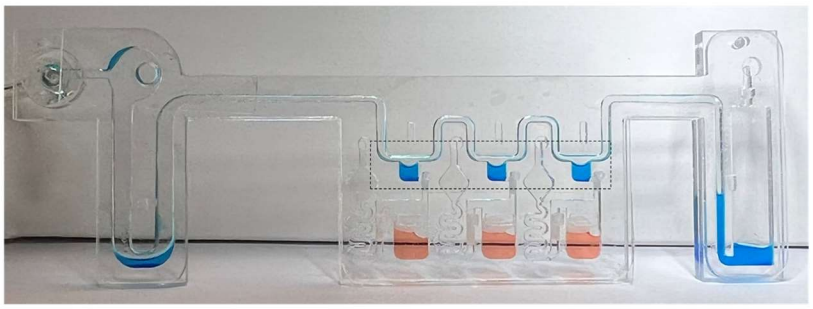


Figure S3. Mobile phone user interface and result unloading. The Android SLIDE application guides the user through steps to set up and run the test. (a-b) Collect user information. (c-e) Instructions for the user to collect samples. (f-h) Selection of the Bluetooth and the test initiation. (i) Real-time analysis during the test. (j) Upload the testing result to local or Google drive.

(a)



(b)



(c)

	Trapped Area (mm^2)	PMMA Thickness (mm)	Volume (µl)
Trap 1	6.4	1.6	10.24
Trap 2	6.2	1.6	9.92
Trap 3	6.5	1.6	10.40

Figure S4. The trapping volume characterization. (a) The original color image of the trapping result. (b) The processed image by ImageJ for trapping area analysis. The black-white inverted image of the 8-bit image. An upper threshold of the grayscale was applied to the black-white image. (c) The trapping result of three chambers. The average trapping volume among three-chamber is 10.25 µl. The blue liquid is the Saliva with the blue dye. The orange liquid is the RT-LAMP master mix with orange dye for better visualization.

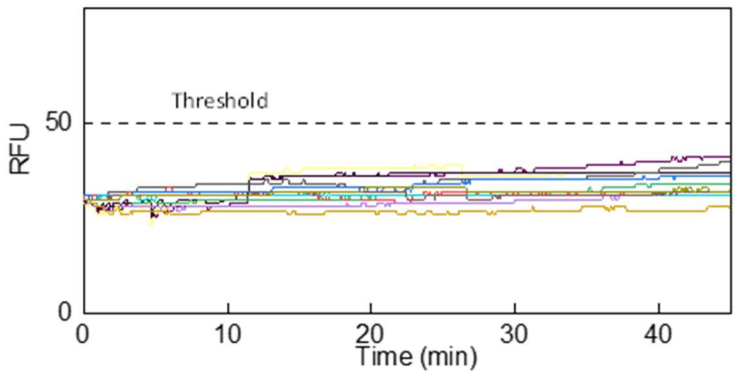


Figure S5. Representative measurement for 9 negative control samples. All of the samples were measured for 45 minutes. The baseline varies from RFU 28 to 42. Therefore, we set the RFU 50 as the threshold to avoid false positives.

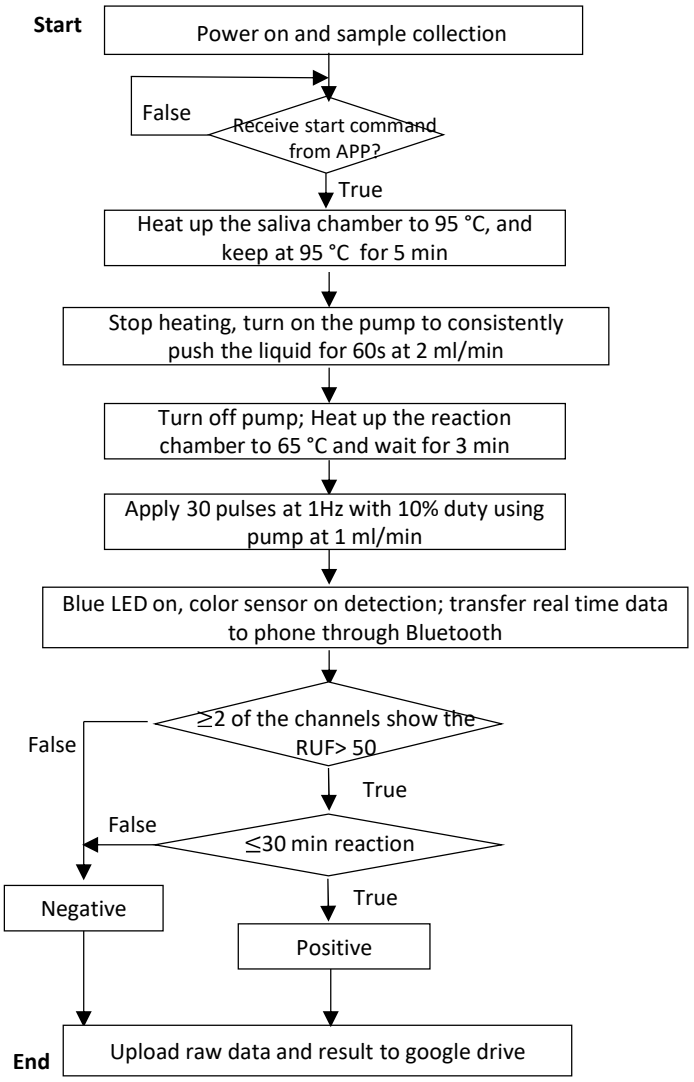


Figure S6. The Flow chart of the automatic workflow from sample collection, sample preparation, sample dispensing, RT-LAMP reaction, and the result storage and communication.

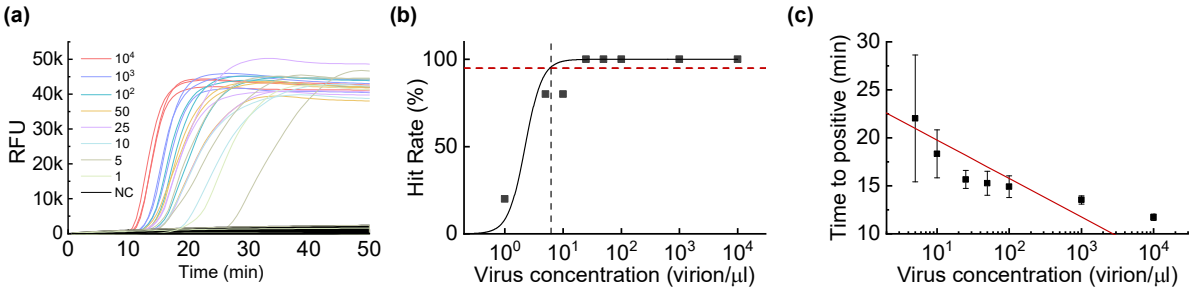


Figure S7. (a) Real-time RT-LAMP results with different concentrations of the spiked saliva samples (1 copies/µl to 10^4 copies/µl) using a benchtop PCR machine. The spiked saliva samples were manually prepared in the heating block for 5 min at 95°C. 10 µl of the sample was taken by pipette and transferred into the 30 µl of the RT-LAMP master mix. (b) The extracted hit rate at various virus particle concentrations to establish LoD. (c) The inversely proportional relationship between the time to positive and virus particle concentration.

Supplementary Tables

Table S1. RT-LAMP primer set targeting N regions of SARS-Cov-2[1-2]

Primer		Sequence (5'-3')
Gene N	**F3**	AACACAAGCTTTCGGCAG
	B3	GAAATTTGGATCTTTGTCATCC
	BIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC
	FIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
	LF	TTCCTTGTCTGATTAGTTC
	LB	ACCTTCGGGAACGTGGTT

Table S2. RT- LAMP Master mix

Components	Working Concentration (40µl)
FIP/BIP primers	1.6 µM
F3/B3 primers	0.2 µM
LF/LB primers	0.4 µM
Isothermal Amplification	1x
$MgSO_4$	6 mM
Betaine	0.4 M
dNTP	1.4 mM
syto-9 green fluorescent	10 µM
Bst 2.0 DNA polymerase	10 U
WarmStart Reverse Transcriptase	7.5 U
Saliva sample	10 µl
UP Water	1 µl

Table S3. Materials for analyzer

System	Vendor	Description	Part#	Function
Electronics	Adafruit	Arduino Nano	A000005	Microcontroller
Electronics	Newark	Through Hole Resistor, 10 kΩ	38K0328	Temperature control
Electronics	Newark	Through Hole Resistor, 47 Ω	38K0326	Resistors for LED
Electronics	Newark	Capacitor 470uF	65R3137	Power Stabilizing
Electronics	Newark	Capacitor 0.33uF	46P6304	Voltage regulating
Electronics	Newark	Capacitor 0.1uF	46P6667	Voltage regulating
Electronics	DigiKey	8 Position Header Connector	S7041-ND	PCB connector
Electronics	Adafruit	Jumper Wire	1568-1512-ND	Wiring
Electronics	DigiKey	Tactile switch	1825910-6	Push-button
Electronics	Adafruit	Bluetooth Low Energy (BLE)	2633	Bluetooth module
Electronics	ZIPPY	1300mAh 2S 25C Lipo Pack	ZC.1300.2S.25	Battery
Pumping	Servoflo	Piezo-pump	mp6-hyb	Drive reagents
Pumping	Servoflo	Driver circuit	mp6-oem	Power the piezo-pump
PCB fabrication	OSH Park	Control PCB	NA	
PCB fabrication	OSH Park	LED PCB	NA	
PCB fabrication	OSH Park	Optical sensor PCB	NA	
Thermal	Newark	N Channel Power MOSFET	63J7707	Switch for resistive heater
Thermal	Newark	Thermistor	95C0606	Temperature sensing
Thermal	DigiKey	Power resister	MP725-5.00	Heating the alumin block
Thermal	Mc master	Aluminum	9246K523	Heating block
Optics	Adafruit	Color Sensor	TCS34725	Detection
Optics	Newark	LED, Blue, T-1 3/4 (5mm)	04R6674	Fluorescence excitation
Enclosure	Inhouse	3D printed platform	NA	NA

Supplementary Videos

Supplementary Video S1. (Duration: 29 s) Representative cases of the automatic sample dispense on the cartridge.

Supplementary Video S2. (Duration: 79 s) Workflow of the saliva-based SARS-CoV-2 self-testing with RT-LAMP.

Supplementary References

(S1) Tang, Z.; Nouri, R.; Dong, M.; Yang, J.; Greene, W.; Zhu, Y.; Yon, M.; Nair, M. S.; Kuchipudi, S. V.; Guan, W. Rapid detection of novel coronavirus SARS-CoV-2 by RT-LAMP coupled solid-state nanopores. *Biosensors and Bioelectronics* **2022,** *197*, 113759.
(S2) Broughton, J. P.; Deng, X.; Yu, G.; Fasching, C. L.; Servellita, V.; Singh, J.; Miao, X.; Streithorst, J. A.; Granados, A.; Sotomayor-Gonzalez, A. CRISPR–Cas12-based detection of SARS-CoV-2. *Nature biotechnology* **2020,** *38* (7), 870-874.