Compact Multiplex PCR Device for HIV-1 and HIV-2 Viral Load Determination from Finger-Prick Whole Blood in Resource-Limited Settings

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Supplementary Text

Real-time PCR Portable PCR analyzer instrumentation

The complete design of the device was developed using PTC Creo software, with the casing produced through 3D printing on a METHOD X, MakerBot printer. The printed circuit board (PCB) was designed using Autodesk Eagle and manufactured by OSH Park. Assembly of the electronic components and the microcontroller unit on the PCB was performed manually in our laboratory. A resistive heating element (PWR263S-20-2R00J, sourced from Digi-Key) was affixed to the underside of a specially fabricated aluminum heating plate with thermal paste (AATA-5G, Artic Alumina). For accurate temperature control, a thermistor (95C0606, Digi-Key) was integrated at the center of the heating plate, facilitating real-time temperature monitoring. Temperature regulation during nucleic acid testing was achieved through a negative thermal feedback mechanism using an N-channel power MOSFET (63J7706, Digi-Key). The control software for the graphical user interface (GUI) was developed in Android IDE, enabling device management and data exchange via Bluetooth connectivity.

Multiplex RT-qPCR reaction

In this study, we employed a dual-enzyme, one-step RT-qPCR method for detecting HIV-1. The reaction mixture, with a total volume of 20 µL, consisted of 5 µL of TaqMan Fast Virus 1-Step Master Mix, paired with forward and reverse primers (each at a 1 µM concentration), a probe (1 µM), 1 µL of RNA templates, and 11 µL of PCR-grade water. The decision to use 20 µL as the reaction volume was based on the position of the sensor and the sensitivity of the optical detection system. Reducing the volume further could lead to the liquid level falling below the detection plane and insufficient fluorescent signal detection, thus affecting the accuracy of the results. We used a verified set of HIV-1 RT-qPCR primers for this assay(Henrich et al., 2012). The RT-qPCR protocol followed specific thermal conditions, beginning with a single reverse transcription step at 50 ℃ for five minutes to convert HIV-1 RNA into cDNA. This step was immediately followed by a denaturation phase at 95 ℃ for 20 seconds, and then 40 cycles of amplification. Each amplification cycle consisted of a 3-second denaturation at 95 ℃ and a 30-second annealing and extension phase at 60 ℃. The details of the primers and probes are listed in Supplementary Tables S3, S4, and S5. In our experiment, the reporters used were ATTO425, HEX, and Cy5.

Supplementary Figures

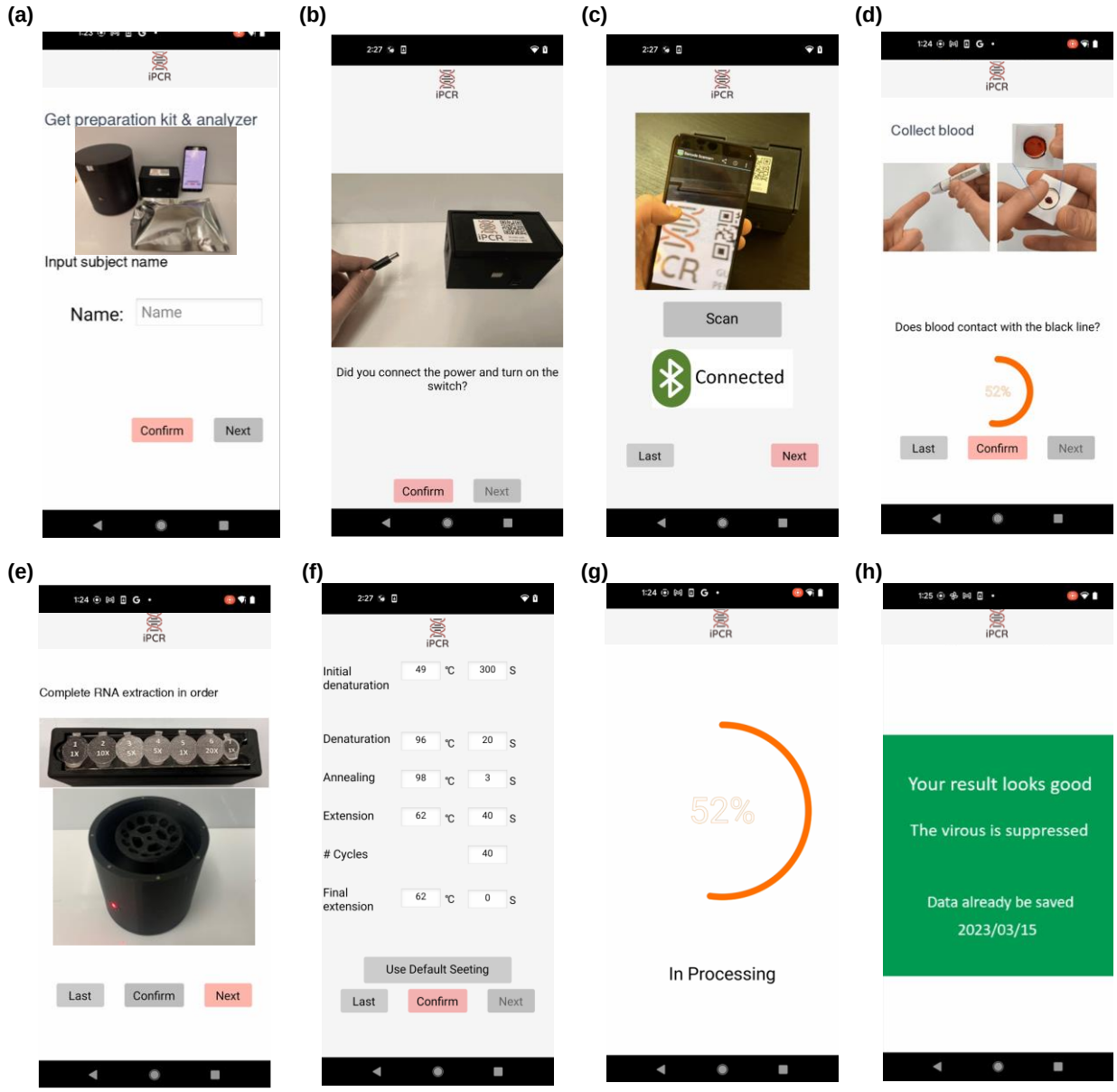


Figure S1. Mobile Application Interface: The Android iPCR app provides step-by-step guidance for setting up and conducting the test. **(a)** Enter user information. **(b-c)** Power on the analyzer and establish a Bluetooth connection. **(d-e)** Follow instructions for sample collection. **(f)** Input PCR reaction parameters in real time. **(h)** Perform real-time analysis during the test and upload results to local storage or Google Drive.

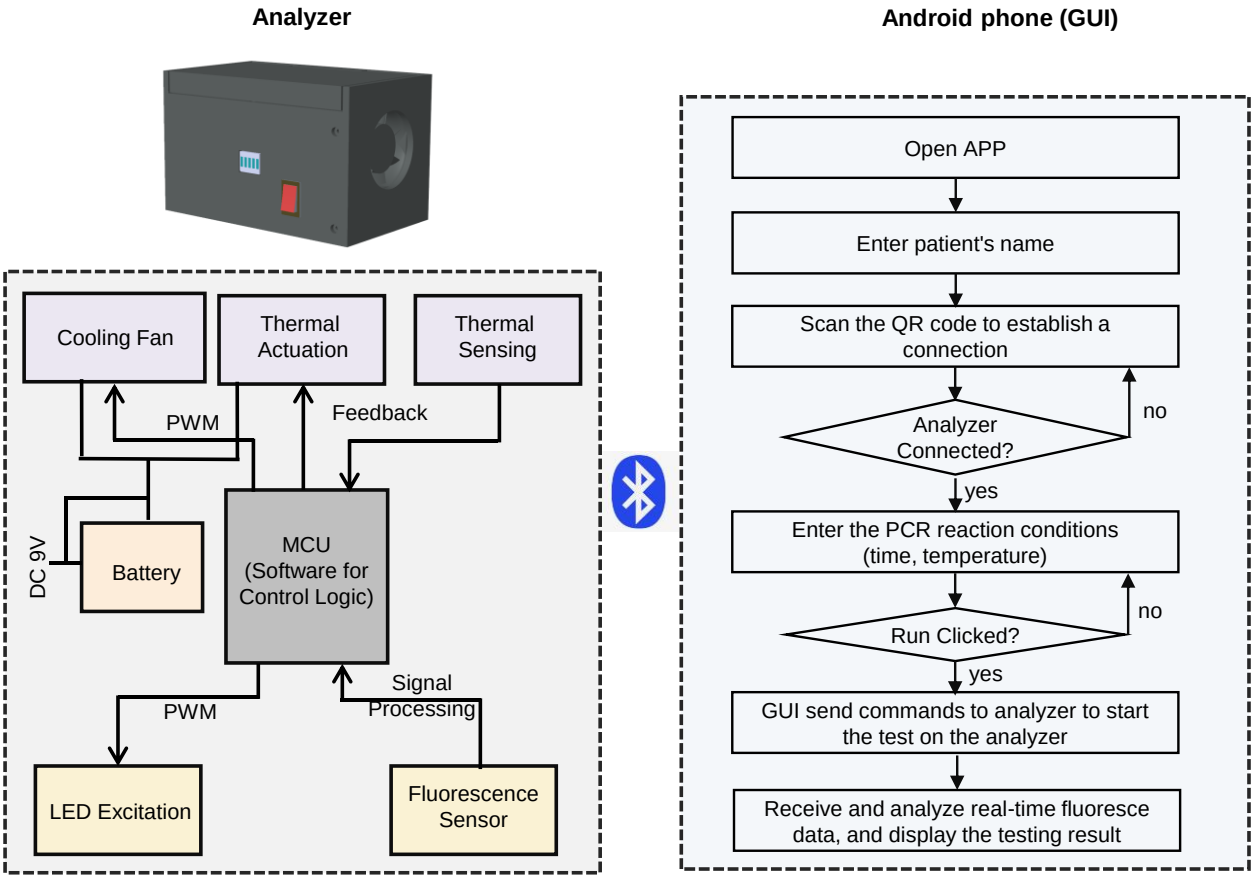


Figure S2. A streamlined block diagram illustrates all components of the device. Outputs or actuators include a fan, a resistive heater, an LED for excitation, and a status LED bar. Inputs for feedback comprise a thermistor and an RGB color sensor. At the heart of the system, an Arduino Nano orchestrates the interactions among these modules. The device interfaces with an Android phone through a Bluetooth module, managed via the Arduino IDE. Amplification data is captured in real time, then processed and analyzed offline. This setup has the potential to be integrated with a specifically designed graphical user interface (GUI) for Android, alongside the development process for the Android GUI.

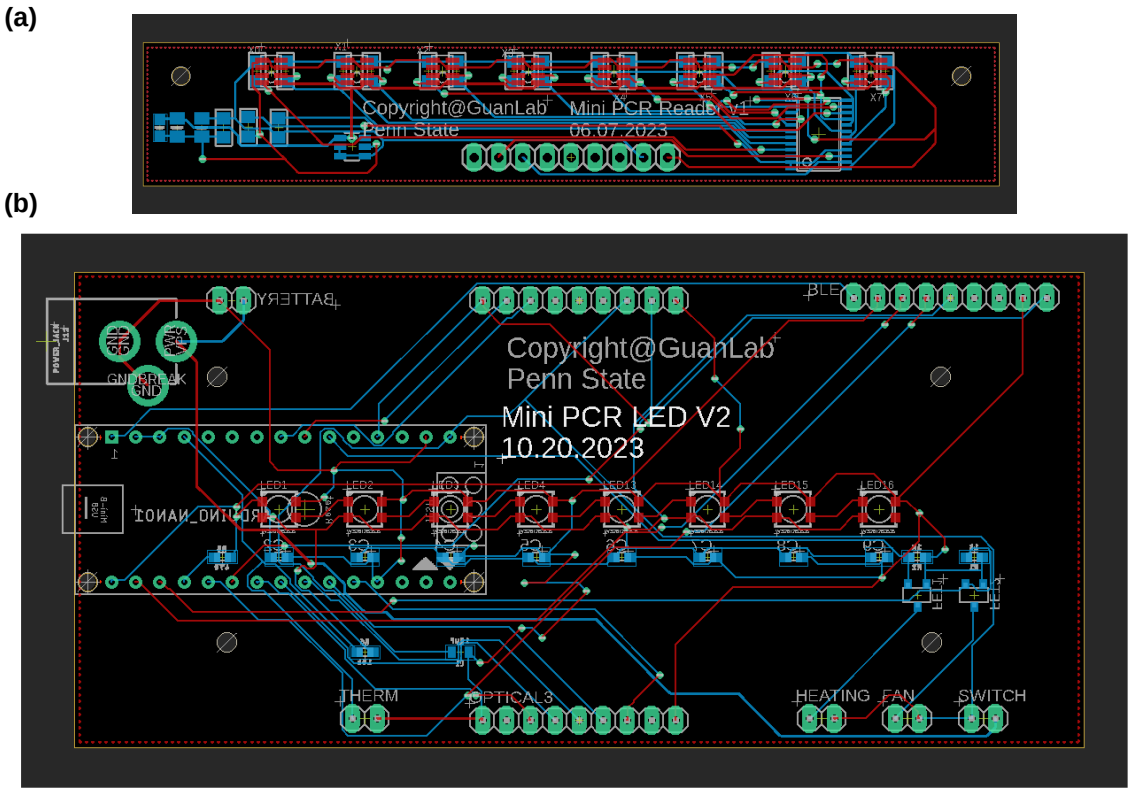


Figure S3. PCB Circuit Design. (a) The PCR design for the optical module primarily focuses on integrating four optical sensors and their corresponding components. (b) The motherboard design incorporates an MCU, a Bluetooth communication module, an excitation light source, and a temperature control unit.

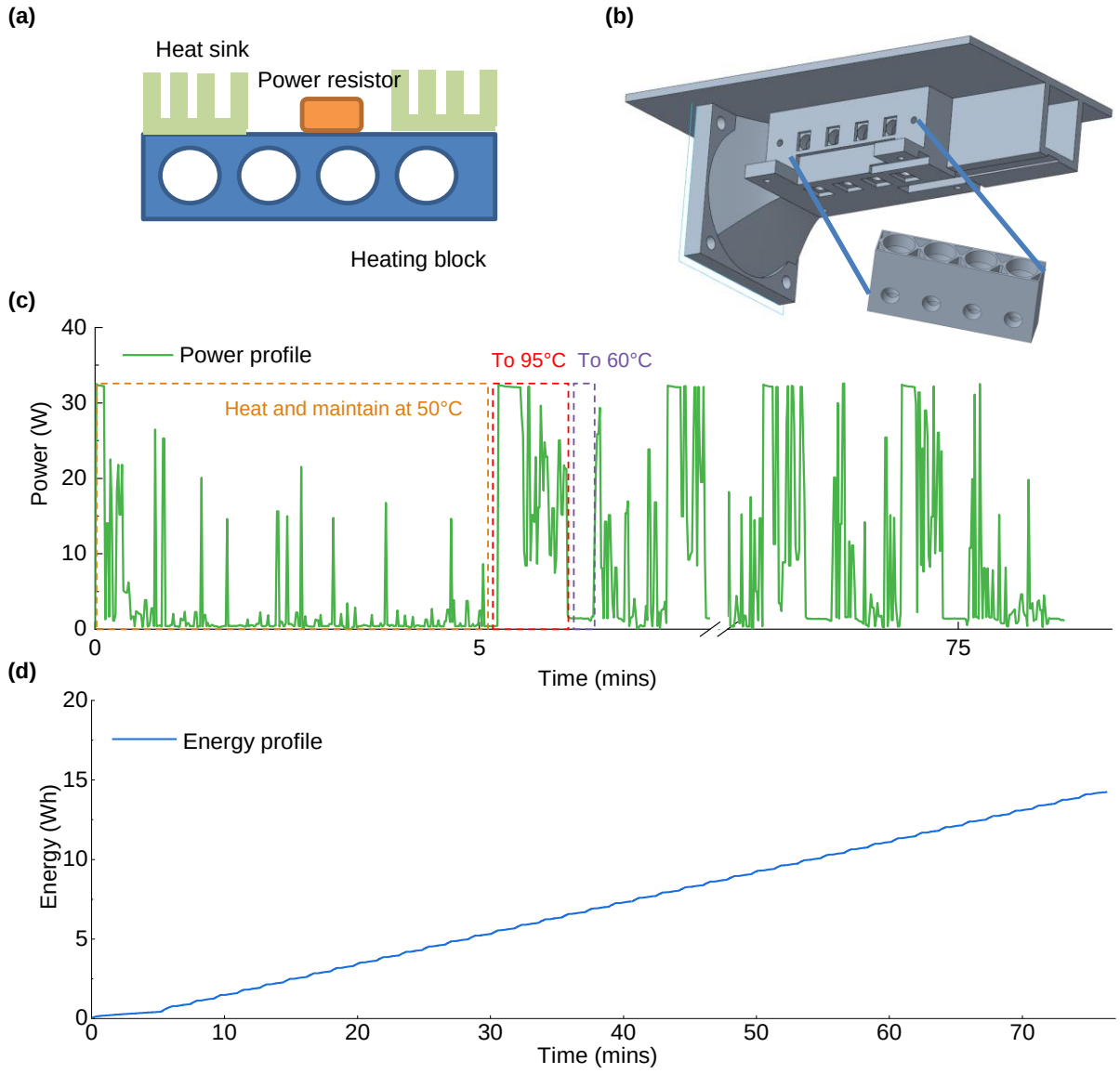


Figure S4. (a) Schematic of the thermal module. The thermal module consists of a power resistor as the heat source, a thermistor for temperature feedback, and a CNC-machined aluminum plate designed with hollow sections to reduce weight and enhance heating and cooling efficiency. **(b)** The heating block within the thermal module is positioned in a duct made from ABS plastic, with a fan installed at one end of the duct. The combination of the fan, duct, and power resistor enables rapid heating and cooling. **(c)** Power profile during a 77-minute test cycle. The profile is divided into three sections: the first (yellow) section shows heating from room temperature to 50°C and maintaining this for 5 minutes, the second (red) section represents heating from 50°C to 95°C, and the third (purple) section details cooling from 95°C to 60°C. This is followed by repeated heating and cooling cycles between 95°C and 60°C. **(d)** Energy consumption profile for the entire 77-minute test cycle.

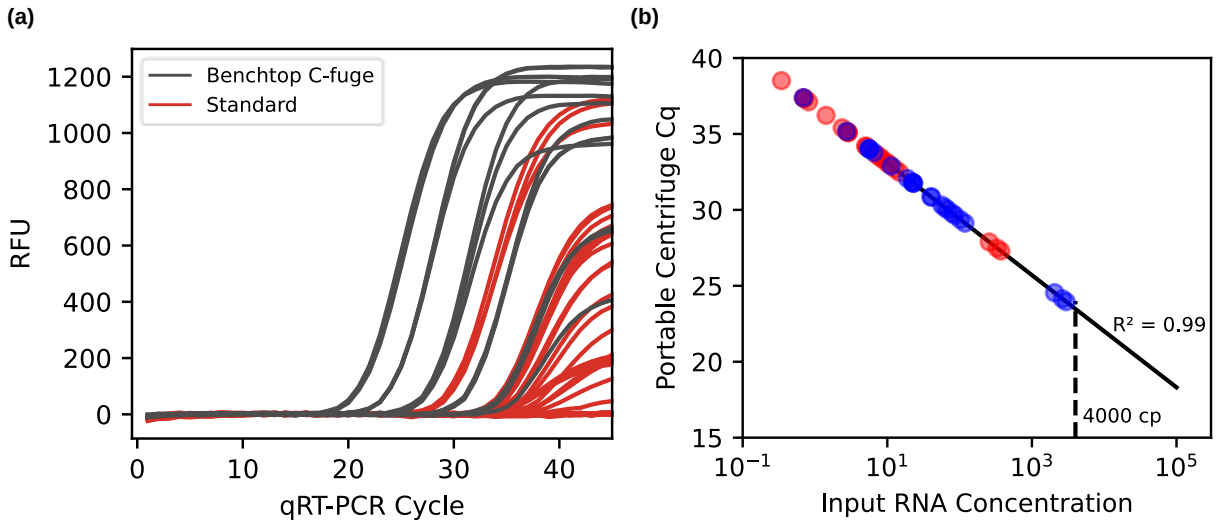


Figure S5. (a) Real-time HIV-1 RT-qPCR amplification data: the black line represents the reference reaction with known copy numbers (10^6, 10^5, 10^4, 10^3, 10^2, 10), and the red line is RNA extracted using the lab-based protocol. **(b)** RT-qPCR reference line to quantify extracted RNA: blue dots are the reference reaction with known copy numbers, and red dots are RNA extracted using the lab-based protocol.

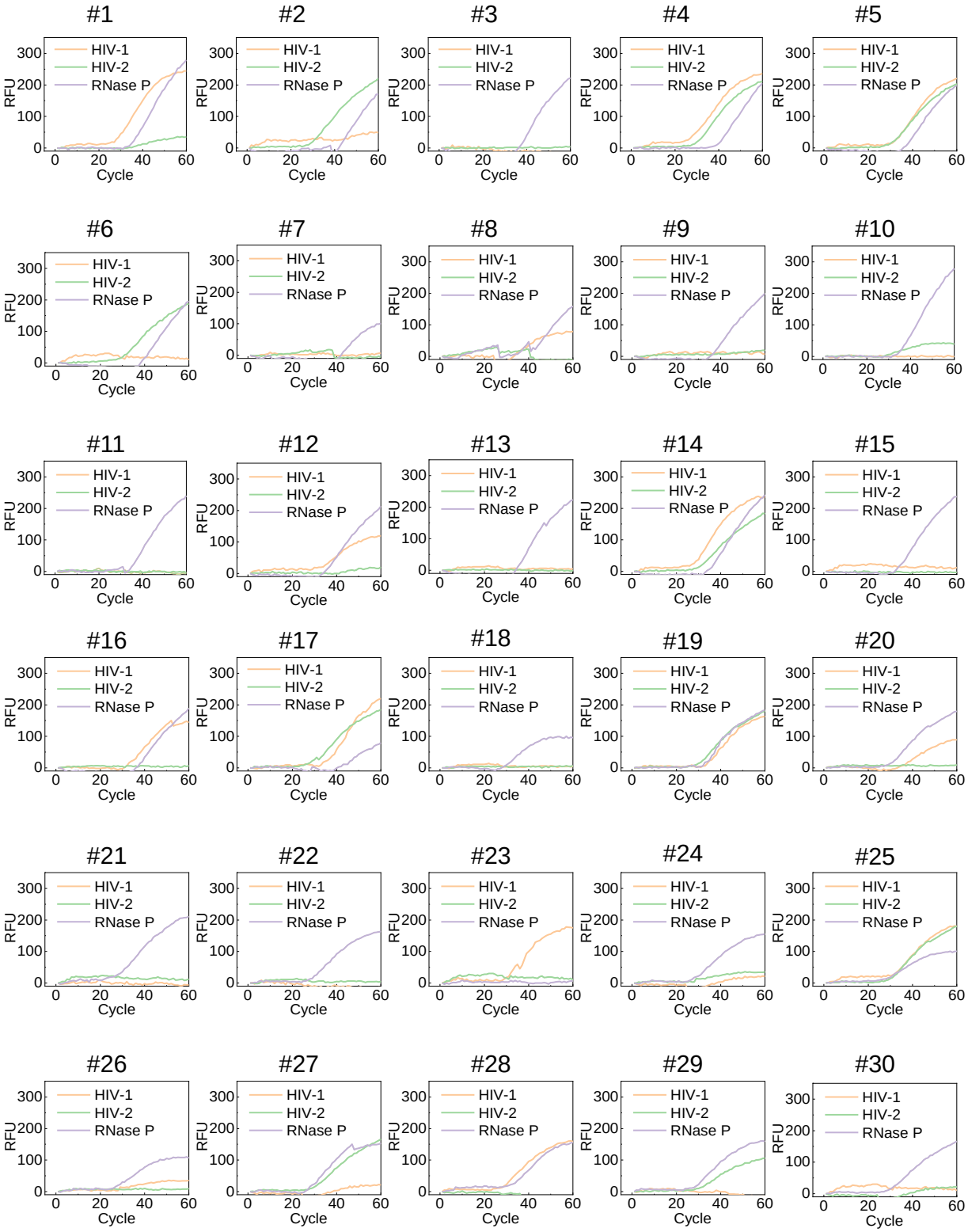


Figure S6. The real-time RT-PCR curves for 30 clinical samples tested with our device are presented. Each set includes curves for HIV-1, HIV-2, and RNase P from three separate optical channels.

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Expect (Cycles) or (P/N)										
HIV-1	27.88	N	N	27.48	33.35	N	N	34.17	N	N
HIV-2	N	29	N	29	29	29	N	N	N	N
Rnase P	P	P	P	P	P	P	P	P	P	P
Resule (Cycles) or (P/N)										
HIV-1	28	N	N	28	32	N	N	36	N	N
HIV-2	N	29	N	30	32	33	N	N	N	32
Rnase P	P	P	P	P	P	P	P	P	P	P

	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
Expect (Cycles) or (P/N)										
HIV-1	N	35.17	N	27.29	N	34.21	35.09	N	33.48	36.23
HIV-2	N	N	N	29	N	N	29	N	29	N
Rnase P	P	P	P	P	P	P	P	P	P	P
Resule (Cycles) or (P/N)										
HIV-1	N	37	N	28	N	33	36	N	35	36
HIV-2	N	49	N	30	N	N	27	N	30	N
Rnase P	P	P	P	P	P	P	P	P	P	P

	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30
Expect (Cycles) or (P/N)										
HIV-1	N	N	32.7	N	32.99	N	N	32.47	N	N
HIV-2	N	N	N	N	29	N	29	N	29	N
Rnase P	P	P	P	P	P	P	P	P	P	P
Resule (Cycles) or (P/N)										
HIV-1	N	N	31	N	34	N	N	32	N	N
HIV-2	N	N	N	N	31	N	32	N	31	N
Rnase P	P	P	N	P	P	P	P	P	P	P

Figure S7. The quantification cycle (Cq) values for HIV-1 and HIV-2 from **Figure 4**, obtained using benchtop PCR, are presented as expected cycles. The clinical samples also contain RNase P, indicated as positive ("P") in the expected positive/negative (P/N) values. The data from **Figure S6** are used to convert these findings into Cq values for HIV-1, HIV-2, along with their respective positive or negative conclusions for RNase P.

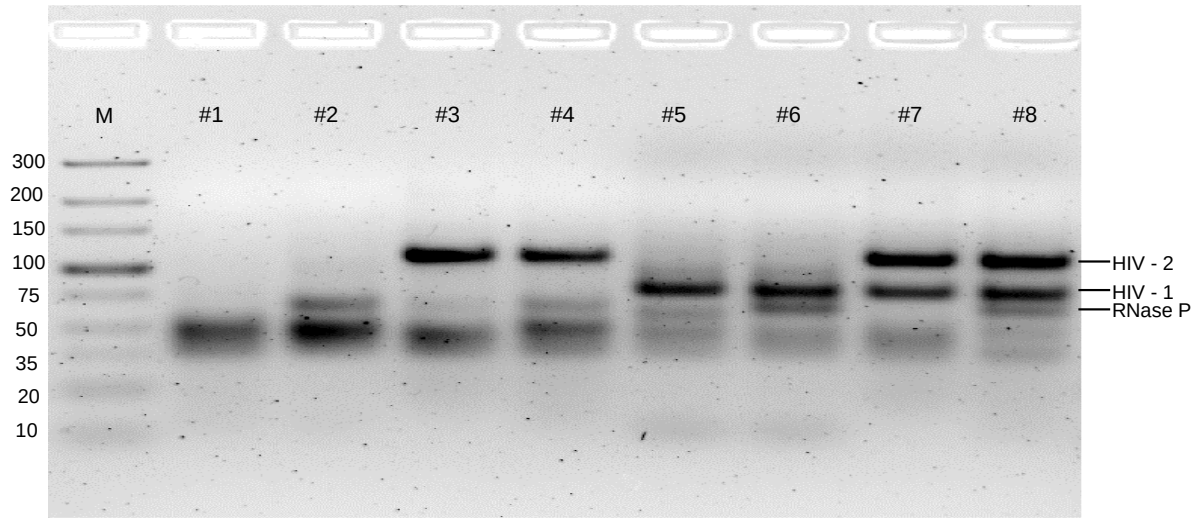


Figure S8. Agarose gel electrophoresis was utilized to examine the RT-PCR products, providing a complete image showcasing the amplification profiles for Samples 1 through 8.

Supplementary Tables

Table S1. Reverse-transcriptase sub-region targeting HIV-1

Sequence (5' → 3')
tctcgacgcaggactcggcttgctgaagcgcgcacggcaagaggcgaggggcggcgactggtgagtacgccaaaaattttgactagcggaggct agaaggagagagatgggtgcgagagcgtcagta

Table S2. Reverse-transcriptase sub-region targeting HIV-2

Sequence (5' → 3')
gcgcgagaaactccgtcttgcgagggaaaaaagcagatgaattagaaacaattaggttacggcccggcgggaagaaaaagta cagactaaaacatattgtgtgggcagcgaa

Table S3. RT-qPCR primer set targeting HIV-1

Primer	Sequence (5' → 3')
Forward	TACTGACGCTCTCGCACC
Reverse	TCTCGACGCAGGACTCG
Probe	/5ATTO425N/CCACCCCAC/ZEN/AAGATTTAAACACCATGCTAA/3IABkFQ/

Table S4. RT-qPCR primer set targeting HIV-2

Primer	Sequence (5' → 3')
Forward	GCGCGAGAAACTCCGTCTTG
Reverse	TTCGCTGCCCACACAATATGTT
Probe	/5HEX/TAGGTTACG/ZEN/GCCCGGCGGAAAGA/3IABkFQ/

Table S5. RT-qPCR primer set targeting Rnase P

Primer	Sequence (5' → 3')
Forward	AGATTTGGACCTGCGAGCG
Reverse	GAGCGGCTGTCTCCACAAGT
Probe	/5Cy5/TTCTGACCT/TAO/GAAGGCTCTGCGCG/3IAbRQSp/

Supplementary Table S6. Bill of materials of the analyzer

System	Description	Part#	Function	Unit Cost ($)	Unit Qty.	Ext. Cost ($)
Electronics	Arduino Nano	7630049200173	Microcontroller	6.00	1	6.00
Electronics	Adafruit Bluefruit LE SPI	2633	Bluetooth Low Energy	14.00	1	14.00
Electronics	Capacitor 10uF	P10425CT	Power stabilizing	0.147	1	0.15
Electronics	DC Barrel Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	36-pin stripe male header	392	Headpins	4.95	0.1	0.50
Thermal	Aluminum CNC block	CP-0.91-0.91	Heating Stage	5.75	0.25	1.44
Thermal	2Ω Power Resistor	MP725-2.00-FCT	Heater	7.01	1	7.01
Thermal	SMD Resistor, 1kΩ	311-1.00KHRCT	Temperature/EM control	0.0072	1	0.01
Thermal	Thermistor	235-1057	Temperature sensing	3.5	1	3.50
Thermal	SMD Resistor, 10kΩ	RNCP0603FTD10K0CT	Temperature control	0.1	1	0.10
Thermal	N Channel Power MOSFET	DMN1019USN-7DICT	Switch for heater and fan	0.354	2	0.70
Thermal	FAN AXIAL 40X15MM 12VDC	603-1006-ND	Fan for colling	8.86	1	8.86
Optics	Res Cermet Trimmer 500K ohm	3296W-1-501RLF	Adjust LED intensity	0.6	3	1.80
Optics	SMD Resistor, 1kΩ	RNCP0805FTD1K0CT	Optical module	0.0277	3	0.08
Optics	SMD Resistor, 10kΩ	RNCP0805FTD10K0CT	Optical module	0.0277	8	0.22
Optics	IC Color Sensor	AS7341	Optical detection	3.00	3	9.00
Optics	SMD LED, Blue	1497-1138-1	Fluorescence excitation	0.528	3	1.58
Optics	Capacitor 10uF	P10425CT	Power stabilizing	0.147	3	0.44
Optics	Capacitor 0.1uF	P01425CT	Power stabilizing	0.147	1	0.15
Optics	Voltage Regulator	AP7312-3.3/1.8	Power supplier	0.8	1	0.80
Optics	I2C Multiplexer	TCA9548A	I2C expender for sensor	1.10	1	1.10
Enclosure	ABS Filament (Black)	-	3D platform material	69.00	0.05	3.45
Enclosure	Screws (M2 cap screw)	91290A015	Assembly	0.14	10	1.40
			Total Cost			**$63.54**

Table S7. Cost per test

Reagents	Vendor	Function	Stock Vol (mL)	Unit Cost ($)	Volume (μL)/rxn	Ext Cost/rxn
RT-LAMP Mixes	Meridian Life Science	LAMP master mix	5	198	6.25	0.2475
HIV-1 Forward	IDT	PCR master mix	0.856	12.05	0.4	0.00563
HIV-1 Reverse	IDT	PCR master mix	0.67	9.15	0.4	0.00546
HIV-1Probe	IDT	PCR master mix	0.185	381	0.4	0.82378
HIV-2 Forward	IDT	PCR master mix	0.697	9.64	0.4	0.00553
HIV-2 Reverse	IDT	PCR master mix	0.87	10.60	0.4	0.00487
HIV-2Probe	IDT	PCR master mix	0.201	311	0.4	0.61891
RnaseP Forward	IDT	PCR master mix	0.763	9.15	0.4	0.0048
RnaseP Reverse	IDT	PCR master mix	0.68	9.64	0.4	0.00567
RnaseP Probe	IDT	PCR master mix	0.231	284	0.4	0.49177
Lysis Buffer	Invitrogen	Sample Prep.	25	6	200	0.02
Wash Buffer	Invitrogen	Sample Prep.	37.5	54	500	0.12
Elution Buffer	Invitrogen	Sample Prep.	11	35	30	0.1
Ethanol	Sigma-Aldrich	Sample Prep.	100	10	200	0.02
Material	**Vendor**	**Function**	**Stock Vol (sq. m.)**	**Unit Cost ($)**	**Volume (sq. mm)/rxn**	**Ext Cost/rxn**
Vivid PSM-GR	Pall Corporation	PSM card	0.06	30.00	200	0.30
Silica column	Qiagen	RNA extraction		2.00	1	2.00
PCR tube		Fisher scientific		0.01	1	0.01
					Total	**$4.28**

Supplementary Code

Supplementary Code S1. Arduino and Android Code.

Supplementary Reference

Henrich, T.J., Gallien, S., Li, J.Z., Pereyra, F., Kuritzkes, D.R., 2012. Journal of Virological Methods 186, 68–72.