A Handheld Purification-Free Nucleic Acid Testing Device for Point of Need Detection of Malaria from Whole Blood

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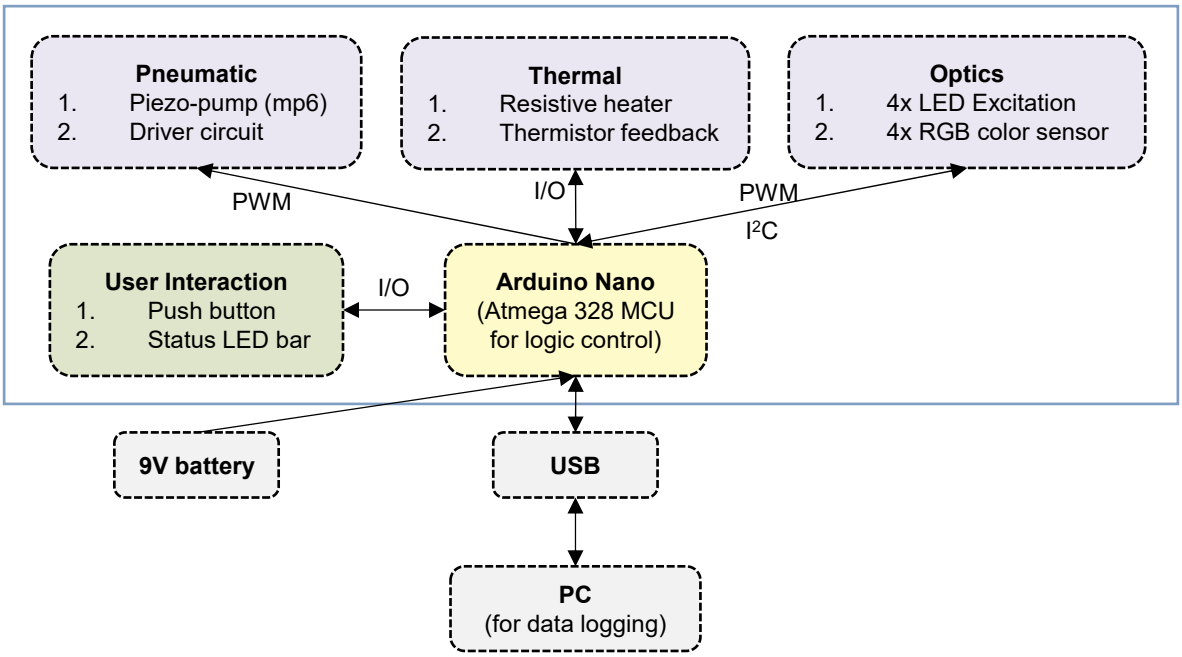


Figure S1. A simplified block diagram of the instrument shows all the modules. The piezo pump, resistive heater, LED excitation, and status LED bar are outputs or actuators, while thermistor feedback, push-button, and RGB color sensor are feedbacks or data inputs. An Arduino Nano is at the center coordinating between all the modules. The instrument communicates with a computer over a serial USB interface via the Arduino IDE. Real-time amplification data is extracted, processed, and plotted offline with future potential for integration with a custom-designed GUI.

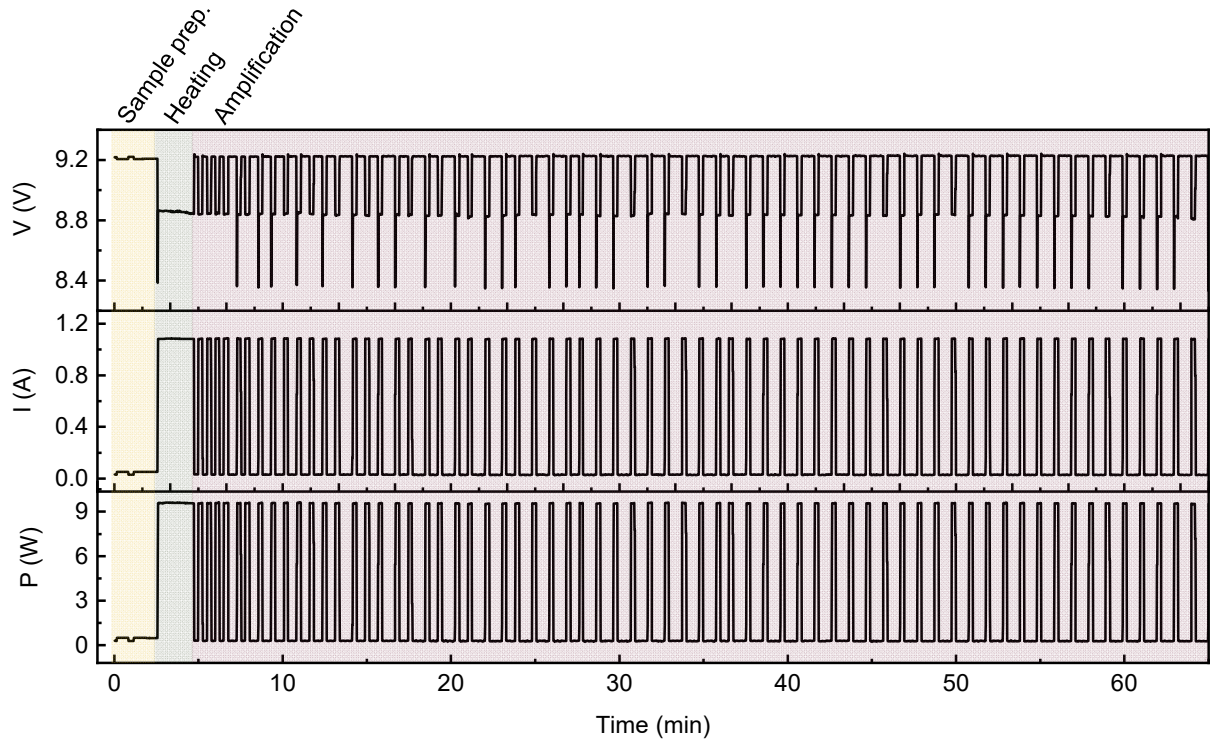


Figure S2. To estimate the instantaneous power consumed by the analyzer, we used a power meter GPM8310 from GW-Instek, Germany. Section 1 represents the sample preparation phase, section 2 represents the heating phase requiring ~1.08A continuous current consumption, and section 3 represents the test's amplification phase, as highlighted in the voltage, current, and power graphs. During the amplification phase, the square-wave pattern results from the heater turning on and off to maintain the necessary temperature during the LAMP reaction. The high current drawn causes a voltage drop across the small internal resistance (mΩ) of the battery and can be seen as the sudden dips in the voltage. The total energy consumed is 3.087 Wh over ~65 minutes.

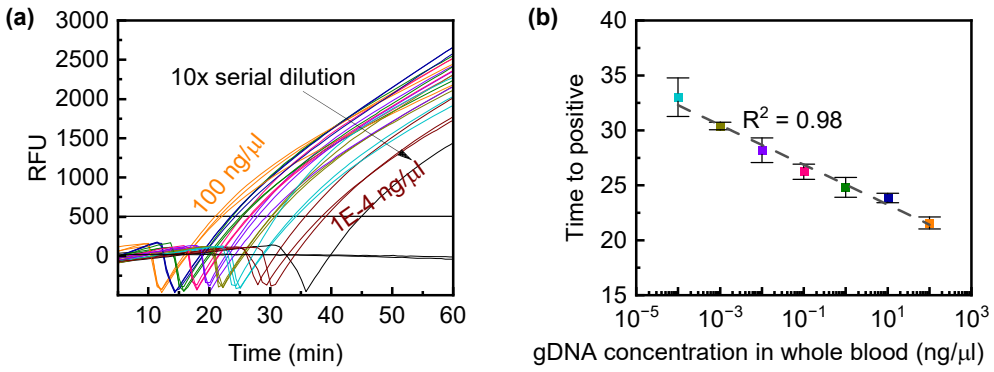


Figure S3. a) Real-time LAMP results mock blood samples spiked with 10^{2} to 10^{-4} ng/µl gDNA (10x serially diluted) using a benchtop thermal cycler. The samples were subject to Arcis reagent-based sample preparation in traditional Eppendorf tube and 1 µl of the prepared blood samples was used in 25 µl LAMP reaction on a benchtop thermal cycler (triplicates). b) The inversely proportional relationship between the time to positive and gDNA concentration.

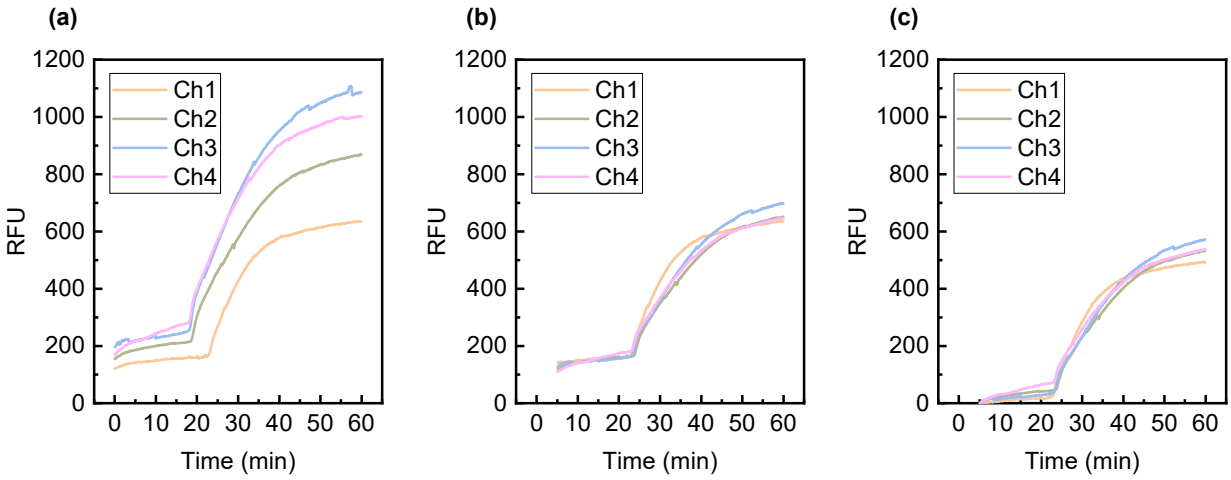


Figure S4. Data processing. a) The raw real-time amplification data, as collected. b) Step 1 is scaling the amplification signals of the 2nd, 3rd, and 4th chambers to the 1st chamber signal as a reference. It must be noted that the values collected over the first five minutes have been ignored since the temperature of the chamber contents is rising over this period (refer to main Figure 3d). c) Step 2 is subtracting the background signal that is acquired just after the first five minutes from every subsequent value.

Supplementary Tables

Table S1. qPCR assay composition to detect *Plasmodium falciparum* [1]

Component	Final Concentration	Volume/rxn
Master mix	1x	12.5 µl
Forward Primer	0.3 µM	0.375 µl
Reverse Primer	0.3 µM	0.375 µl
Probe	0.2 µM	0.25 µl
UltraPure Water		10.5 µl
Mix volume w/o target		24 µl
Sample with target		1 µl
Total reaction volume		25 µl

Table S2. qPCR assay primer and probe sequences

Primer	Sequence (5' → 3')
Forward	CTTTTGAGAGGTTTTGTTACTTTGAGTAA
Reverse	TATTCCATGCTGTAGTATTCAAACACAA
Probe	/5HEX/TGTTCATAA/ZEN/CAGACGGGTAGTCATGATTGAGTTCA/3IABkFQ/

Table S3. qPCR amplification cycle details

Step	No. of Cycles	Temperature	Fast cycling (min: sec)
Polymerase activation	1	95°C	3:00
Amplification	45	95°C	0:05
		55°C	0:30
Cooling down	1	4°C	up to 24 h

Table S4. LAMP assay composition to detect *Plasmodium falciparum* [2]

Component	Final Concentration	Volume/rxn
NEB Isothermal Buffer	1x	2.5 µl
Forward Outer Primer (F3)	0.2 µM	0.25 µl
Backward Outer Primer (B3)	0.2 µM	0.25 µl
Forward Inner Primer (FIP)	1.6 µM	2 µl
Backward Inner Primer (BIP)	1.6 µM	2 µl
Forward Loop Primer (LF)	0.8 µM	1 µl
Backward Loop Primer (LB)	0.8 µM	1 µl
Betaine	0.4 M	2 µl
MgSO4	7 mM	1.75 µl
Calcein	25 µM	0.625 µl
MnCl2	0.75 mM	1.875 µl
dNTP Mix	1.4 mM	3.5 µl
Bst polymerase	0.32 Unit/µl	1 µl
UltraPure Water		1.25 µl
Mix Volume w/o target		21 µl
Sample with target		4 µl
Total Reaction Volume		25 µl

Table S5. LAMP assay primer sequences

Primer	Sequence (5' → 3')
F3	CTCCATGTCGTCTCATCGC
B3	AACATTTTTTAGTCCCATGCTAA
FIP	ACCCAGTATATTGATATTGCGTGACAGCCTTGCAATAAATAATATCTAGC
BIP	AACTCCAGGCGTTAACCTGTAATGATCTTTACGTTAAGGGC
LF	CGGTGTGTACAAGGCAACAA
LB	GTTGAGATGGAAACAGCCGG

Table S6. Bill of materials for the developed instrument

System	Vendor	Description	Part#	Function	Unit cost ($)	Unit qty.	Ext cost ($)
Electronics	Adafruit	Arduino Nano	A000005	Microcontroller	20.07	1	20.07
Electronics	Adafruit	36-pin stripe male header	392	Headpins	4.95	0.083	0.41
Electronics	Sparkfun	DC Barrel Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	Adafruit	Shield Stacking Headers for Arduino	85	Wire Sockets	1.95	0.33	0.64
Electronics	Newark	Through Hole Resistor, 10 kΩ	38K0328	Temperature control	0.09	1	0.09
Electronics	Newark	Through Hole Resistor, 47 Ω	38K0326	Resistors for LED	0.09	4	0.36
Electronics	Newark	Capacitor 470uF	65R3137	Power Stabilizing	0.11	1	0.11
Electronics	Newark	Capacitor 0.33uF	46P6304	Voltage regulating	0.27	1	0.27
Electronics	Newark	Capacitor 0.1uF	46P6667	Voltage regulating	0.354	1	0.354
Electronics	Adafruit	14 pin wire connector	1171	Wiring	1.05	1	1.05
Electronics	Adafruit	14 pin GPIO ribbon Cable	862	Wiring	2.95	1	2.95
Electronics	DigiKey	Bargraph 5-Segment LEDs	XGUGX5D	Status display	1.57	1	1.57
Electronics	DigiKey	Tactile switch	1825910-6	Push-button	0.09	1	0.09
Pumping	Servoflo	Piezo-pump	mp6-hyb	Drive reagents	30	1	30
Pumping	Servoflo	Driver circuit	mp6-oem	Power the piezo-pump	50	1	50
PCB fabrication	OSH Park	Control PCB	NA		17.5	1	17.5
PCB fabrication	OSH Park	Optics PCB	NA		15	1	15
Thermal	Newark	N Channel Power MOSFET	63J7707	Switch for resistive heater	1	1	1.00
Thermal	Newark	Thermistor	95C0606	Temperature sensing	7.34	1	7.34
Thermal	DigiKey	Power resistor	MP725-5.00	Heating the aluminum block	6	4	24.00
Optics	Adafruit	Color Sensor	TCS34725	Detection	2	4	8.00
Optics	Newark	CREE LED, Blue, T-1 3/4 (5mm)	04R6674	Fluorescence excitation	0.21	1	0.21
Enclosure	Inhouse	3D printed platform	NA	NA	NA	1	NA
Enclosure	Thorlabs	Screws (M3 set screw)	SS3M6	For holding color sensor	9.25	0.0006	0.01
				Total Cost			**$182.27**

Table S7. Cost of performing one test

Reagents/ materials	Vendor	Function	Stock Vol (ml)	Unit Cost ($)	Volume (μL)/rxn	Ext Cost/rxn
NEB Isothermal Buffer	NEB	LAMP master mix	6	24	2.5	0.01
F3	IDT	LAMP master mix	1.4	9.22	0.25	0.002
B3	IDT	LAMP master mix	1.5	10.22	0.25	0.002
FIP	IDT	LAMP master mix	1	7.14	2	0.013
BIP	IDT	LAMP master mix	1.4	9.18	2	0.013
LF	IDT	LAMP master mix	1.7	11.86	1	0.007
LB	IDT	LAMP master mix	1.3	8.61	1	0.007
MgSO4	NEB	LAMP master mix	6	20	1.75	0.006
Calcein	Sigma-Aldrich	LAMP master mix	8000	133	0.63	0
MnCl2	Sigma-Aldrich	LAMP master mix	100	62.6	1.88	0.001
Betaine	Sigma-Aldrich	LAMP master mix	1.5	24.25	2	0.032
dNTP Mix	Thermo Fisher	LAMP master mix	3.2	107	3.5	0.117
Bst polymerase	NEB	LAMP master mix	1	264	1	0.264
UltraPure Water	VWR	LAMP master mix	20	91.88	7.25	0.033
Arcis reagent 1	Arcis Biotechnology	Sample Prep.	7.5	250	30	1
Arcis reagent 2	Arcis Biotechnology	Sample Prep.	1	50	37.5	1.87
Mineral oil	Sigma-Aldrich	Sample Prep.	500	51.5	180	0.0001
Pressure-sensitive adhesive tape	Flexcon	Microfluidic cartridge	-	-	4	0.1
Acrylic Sheet	ePlastics	Microfluidic cartridge	-	-	5	0.25
					Total Cost	**$3.73**

Supplementary Video

Supplementary Video S1 (Duration: 86 s). Pressure-driven microfluidic sample preparation of a whole blood sample.

Present Address

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Supplementary References

(S1) Perandin, F.; Manca, N.; Calderaro, A.; Piccolo, G.; Galati, L.; Ricci, L.; Medici, M.; Arcangeletti, M.; Snounou, G.; Dettori, G. Development of a real-time PCR assay for detection of Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale for routine clinical diagnosis. *Journal of Clinical Microbiology* **2004**, *42* (3), 1214-1219.

(S2) Polley, S. D.; Mori, Y.; Watson, J.; Perkins, M. D.; González, I. J.; Notomi, T.; Chiodini, P. L.; Sutherland, C. J. Mitochondrial DNA targets increase sensitivity of malaria detection using loop-mediated isothermal amplification. *Journal of Clinical Microbiology* **2010**, *48* (8), 2866-2871.