Supplementary Information

Sample-to-answer palm-sized nucleic acid testing device towards low-cost malaria mass screening

Gihoon Choi[1], Theodore Prince[1], Jun Miao[2], Liwang Cui[2] and Weihua Guan[1, 3*]

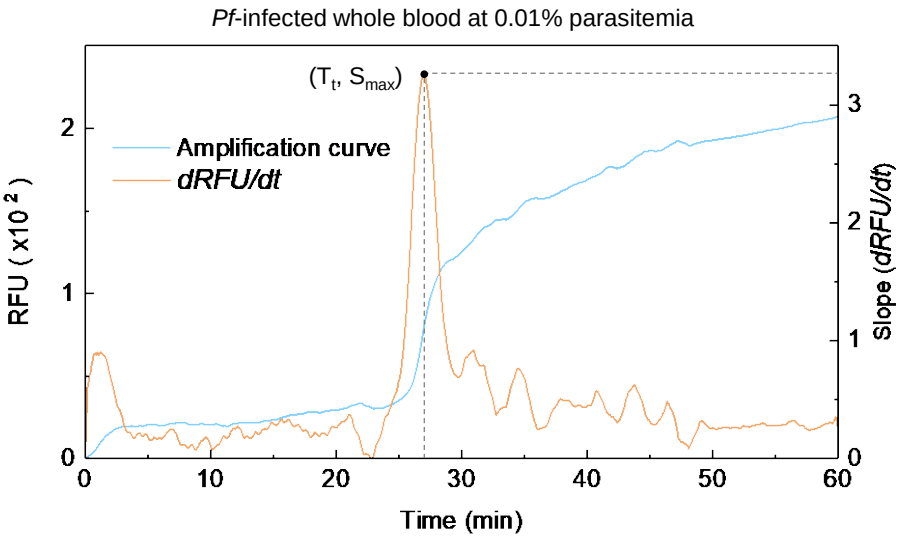
[1] Department of Electrical Engineering, Pennsylvania State University, University Park 16802, USA

[2] Department of Entomology, Pennsylvania State University, University Park 16802, USA

[3] Department of Biomedical Engineering, Pennsylvania State University, University Park 16802, USA

* Correspondence should be addressed to w.guan@psu.edu

Supplementary Figure



Supplementary Figure S1. Determination of the threshold time (T_t). A real-time amplification curve (blue) and its differential profile (d*RFU*/d*t*, orange). The threshold time (T_t) was determined at the maximum slope of RFU. (T_t: threshold time, *t*: time, S_{max}: maximum value of the slope)

Supplementary Table

Supplementary Table S1. Comparison of NAT POC devices for malaria diagnosis

Assay	Detection Method	Real-time Ability	Automation	Sample Processing	LOD (p/µl)	"Sample-to-Answer" Turnaround Time	Ref.
LAMP	Fluorescence	Yes	Automated	Magnetic bead-based extraction	0.6	<50 minutes	This Work
LAMP	Turbidity	No	Manual	gravity-driven filtration	2	< 1 hours	(Lucchi et al., 2016)
LAMP	Turbidity	No	Manual	thermal lysis centrifugation	5	60 - 80 minutes	(Sema et al., 2015)
LAMP	Fluorescence	Yes	Manual	gravity-driven filtration	5	45 minutes	(Xu et al., 2016)
LAMP	Hydroxynaphthol blue (HNB)	No	Manual	Saponin-chelex lysis	1-5	Not reported	(Britton et al., 2015)
PCR	Fluorescence	Yes	Manual	Off-chip	5	< 1 hours	(Nair et al., 2016)
PCR	Fluorescence	Yes	Manual	Off-Chip	2	Not reported	(Taylor et al., 2014)
RPA	Interferometer	Yes	Manual	Dimethyl adipimidate/thin film extraction	1	~1.2 hours	(Liu et al., 2016; Shin et al., 2015)
HDA	Lateral flow strip	No	None	None	200	~2.5 hours	(Li et al., 2013)

Supplementary Table S2. Primer sets for genus-, *Pf*-, and *Pv*-specific LAMP amplification.

Species	Primer	Sequence (5' → 3')
Plasmodium genus(Polley et al., 2010)	F3	TCGCTTCTAACGGTGAACT
	B3c	AATTGATAGTATCAGCTATCCATAG
	FIP (F1c – F2)	GGTGGAACACATTGTTTCATTTGATCTCATTCCAATGGAACCTTG
	BIP (B1 – B2c)	GTTTGCTTCTAACATTCCACTTGCCCGTTTTGACCGGTCATT
	LF	CACTATACCTTACCAATCTATTTGAACTTG
	LB	TGGACGTAACCTCCAGGC
P. falciparum(Polley et al., 2010)	F3	CTCCATGTCGTCTCATCGC
	B3c	AACATTTTTTAGTCCCATGCTAA
	FIP (F1c – F2)	ACCCAGTATATTGATATTGCGTGACAGCCTTGCAATAAATAATATCTAGC
	BIP (B1 – B2c)	AACTCCAGGCGTTAACCTGTAATGATCTTTACGTTAAGGGC
	LF	CGGTGTGTACAAGGCAACAA
	LB	GTTGAGATGGAAACAGCCGG
P. vivax(Britton et al., 2016)	F3	GGTACTGGATGGACTTTATAT
	B3c	GGTAATGTTAATAATAGCATTACAG
	FIP (F1c – F2)	CCAGATACTAAAAGACCAACCCACCATTAAGTACATCACT
	BIP (B1 – B2c)	GCTAGTATTATGTCTTCTTTCACTTAATATACCAAGTGTTAAACC
	LF	GATAACATCTACTGCAACAGG
	LB	CTACTGTAATGCATCTAAGATC

Supplementary Table S3. Bill of materials

System	Description	Part#	Function	Unit Cost ($)	Unit Qty.	Ext. Cost ($)
Electronics	Arduino Mega 2560 R3	DEV-1106	Microcontroller	45.95	1	45.95
Electronics	36-pin Stripe Male Header	392	Headpins	4.95	0.083	0.41
Electronics	DC Barrel Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	Shield Stacking Headers for Arduino	85	Wire Sockets	1.95	0.33	0.64
Electronics	Premium Male/Male Jumper Wires	758	Wires	3.95	0.75	2.96
Electronics	Trimmer Potentiometer, 500Ω	62J1468	LED adjustment	1.98	4	7.92
Electronics	Through Hole Resistor, 10kΩ	38K0328	Temperature control	0.09	5	0.45
Electronics	Through Hole Resistor, 47Ω	38K0326	Resistors for LED	0.09	2	0.18
Electronics	Capacitor 470µF	65R3137	Power stabilizing	0.11	1	0.11
Electronics	Capacitor 0.33µF	46P6304	Voltage regulating	0.27	1	0.27
Electronics	Capacitor 0.1µF	46P6667	Voltage regulating	0.354	1	0.354
Electronics	Diode, Standard, 1A, 50V	78K2043	Diode	0.07	1	0.07
Electronics	26 pin Wire Connector	1171	Wiring	4.95	1	4.95
Electronics	26 pin GPIO Ribbon Cable	862	Wiring	2.95	1	2.95
Servo	Micro Size - High Torque Servo	2307	Actuation of disk	11.95	1	11.95
Magnets	Neodymium Disc Magnet Nickel	58605K33	Holding magnetic beads	2.69	4	10.76
Thermal	Cold Plate	CP-0.91-0.91	Heating stage	5.75	0.25	1.44
Thermal	Peltier Heater	102-1667-ND	Heater	16	1	16.00
Thermal	N Channel Power MOSFET	63J7707	Switch for Peltier heater	1.66	1	1.66
Thermal	Thermistor	95C0606	Temperature sensing	7.34	1	7.34
Optics	Color Sensor	1334	Detection	7.16	4	28.64
Optics	Optical Plastic Light Guide	#02-538	Guiding light	2.55	0.24	0.61
Optics	CREE LED, Blue, T-1 3/4 (5mm)	04R6674	Fluorescence excitation	0.21	1	0.21
Bluetooth	Bluetooth Low Energy (BLE 4.0)	1697	Bluetooth connectivity	19.95	1	19.95
LCD	3.5" TFT 320 x 480	85	touch screen LCD	39.95	1	39.95
SD	MicroSD Card Breakout Board	254	SD module	7.5	1	7.50
Enclosure	Adjustable-Friction Hinge	1791A44	Hinge	6.72	2	13.44
Enclosure	ABS Filament	90003001	3D platform material	18.5	0.4	7.40
Enclosure	Acrylic Sheet, 1/8" Thick, 12" x 24"	8505K12	Holding plates	13.46	0.7	9.42
Enclosure	Screws (M4 cap screw)	W8S038	Hinge holding	3.25	0.04	0.13
Enclosure	Screws (M3 set screw)	SS3M6	For holding color sensor	9.25	0.0006	0.01
			Total Cost			**$244.87**

Supplementary Table S4. Microfluidic reagent disc cost

Reagents	Vendor	Function	Stock Vol (ml)	Unit Cost ($)	Vol.(µl)/test	Ext. Cost ($)/test
UltraPure PCR Water	VWR	LAMP master mix	20	91.88	7.25	0.033
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	1.0	7.14	2.00	0.013
BIP	IDT	LAMP master mix	1.4	9.18	2.00	0.013
LF	IDT	LAMP master mix	1.7	11.86	1.00	0.007
LB	IDT	LAMP master mix	1.3	8.61	1.00	0.007
Calcein	Sigma-Aldrich	LAMP master mix	8000	133.00	0.63	0.000
$MnCl_2$	Sigma-Aldrich	LAMP master mix	100	62.60	1.88	0.001
Betaine	Sigma-Aldrich	LAMP master mix	1.5	24.25	2.00	0.032
dNTP Mix	Thermo Fisher	LAMP master mix	3.2	107.00	3.50	0.117
Bst polymerase	NEB	LAMP master mix	1	264.00	1.00	0.264
NEB Isothermal Buffer	NEB	LAMP master mix	6	24.00	2.5	0.010
$MgSO_4$	NEB	LAMP master mix	6	20.00	1.75	0.006
Lysis Buffer	Invitrogen	Sample Prep.	800	142.00	1000.00	0.178
Purification Buffer	Invitrogen	Sample Prep.	20	28.97	30.00	0.043
Wash Buffer	Invitrogen	Sample Prep.	100	144.84	150.00	0.217
Proteinase K	Invitrogen	Sample Prep.	1	1.45	10.00	0.014
Magnetic Beads	Invitrogen	Sample Prep.	2	2.90	10.00	0.014
Acrylic Adhesive	ePlastics	Compact disc	118	9.69	1.5	0.041
1/32" Acrylic Sheet	ePlastics	Compact disc	-	14.98	-	0.025
1/16" Acrylic Sheet	ePlastics	Compact disc	-	17.72	-	0.030
					Total Cost	**$ 1.07**

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Video S1. Device workflow. 20 µl of finger-prick blood was collected using a capillary tube and lysed in the collection tube filled with 1000 µl of lysis buffer. 180 µl of blood lysate was transferred into each binding chamber of the testing units on the reagent compact disc. After loading the sample, the disc was sealed with PSA tape. The prepared disc was inserted into the mobile analyzer for a streamlined nucleic acid sample preparation (binding, washing, and elution), and amplification process. During the amplification, the fluorescence intensity data were recorded on a non-volatile memory card and displayed on the LCD screen in real-time. After the amplification, the built-in algorithm reports the test results to the user. Users also have an option to receive the results using a smartphone.

Video S2. Streamlined DNA extraction and amplification on the reagent compact disc. It consists of the following three steps: binding, washing, and elution. The negatively charged parasite DNAs first bind to the pH-sensitive charge-switchable magnetic beads at pH 5.0. During the binding process (3 min), the reagent compact disc was rotated back and forth slowly to ensure thorough mixing of the beads and the lysate. The DNA-binding magnetic beads were then transferred to the washing chamber by magnetic actuation. The washing process lasts for 4 min, and the magnetic beads with purified DNAs were further transferred to the reaction chamber, where the LAMP master mix is present. The LAMP master mix has a pH of 8.8, which switches the surface charge of the magnetic beads towards negative. The negatively charged DNAs were therefore repelled off from the magnetic beads and eluted into the master mix. After that, the residual magnetic beads were removed from the reaction chamber, and LAMP reaction is initiated.