Supplementary Information

Field-deployable mobile molecular diagnostic system for malaria at the point of need

Gihoon Choi[1], Daniel Song[2], Sony Shrestha[3], Jun Miao[3], Liwang Cui[3] and Weihua Guan[1,2],*

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

2 Department of Biomedical Engineering, Pennsylvania State University, University Park 16802, USA

3 Department of Entomology, Pennsylvania State University, University Park 16802, USA

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

Supplementary Text

Fabrication procedures of the cartridge disc

The reagent compact disc was designed in AutoCAD 2015 (Autodesk Inc.). The reaction chambers and reagent loading holes were patterned by a laser cutter (Epilog Laser System) with a power of 100%, a speed of 30% (for the top/bottom of 0.8 mm thick) and 60% (for the spacer of 0.8 mm thick), and a frequency of 5000 Hz. The patterned top, spacer, and bottom polymethyl methacrylate (PMMA) layers were initially washed with detergent to remove residues from laser cutting, then laminated with adhesive solvent. The assembled disc was cleaned twice with 2% sodium hypochlorite (NaOCl) and distilled water respectively to eliminate inhibitory substances, which could cause chemical interference.

Thermal modules of the AnyMDx instrument

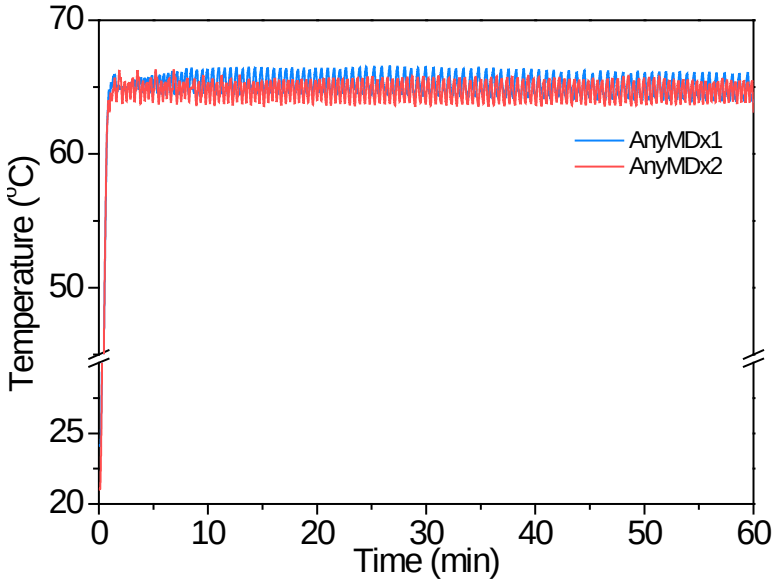
The thermal module consists of an aluminum heating plate, a Peltier heater, and a thermocouple. The Peltier heater was attached to the bottom side of the aluminum heating plate by thermal adhesive to minimize a temperature gradient. A microprocessor controlled feedback system was used to maintain a desired constant temperature (65 °C). For accurate temperature reading, a mini-thermocouple was embedded inside the heating plate. To evaluate the temperature fluctuation, the temperature was monitored for 60 minutes by an external independent thermocouple module (NI-9211, National Instruments). Supplementary **Figure 1** shows the temperature on the aluminum heating plate can reach the set temperature (65 °C) within 40 seconds and continuously maintain a temperature between 64.5 °C to 66.5°C.

Tube validation of nucleic acid preparation with magnetic bead-based method

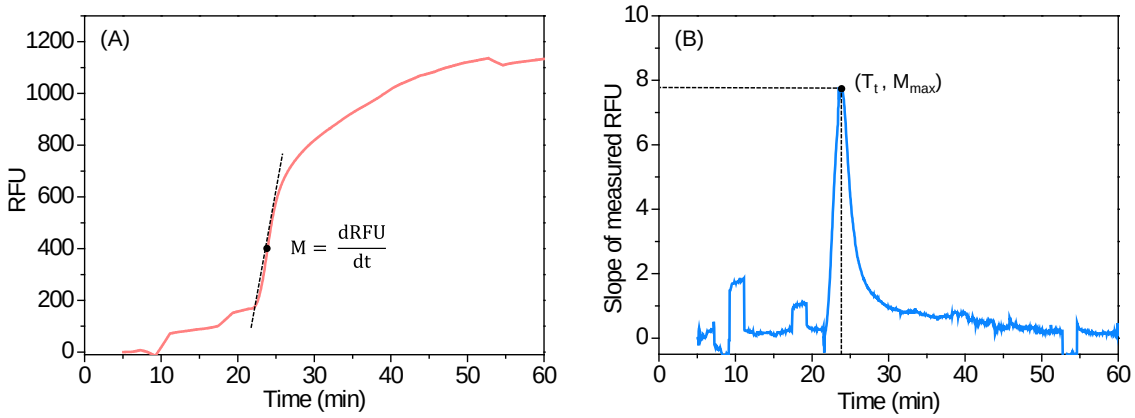
We first performed a reference experiment to confirm compatibility of ChargeSwitch forensic DNA extraction/purification kit. To validate the magnetic bead-based DNA extraction/purification method, we manually carried out tube-level sample preparation by pipetting (**Supplementary Figure 4A**). In step 1, 20 µL of sample (blood) was initially dispensed to the tube, which contains 1 mL of lysis buffer and 10 µL of Proteinase K. This mixture was incubated at room temperature for 2 minutes to lyse the malaria parasites and RBCs. In step 2, 200 µL of purification buffer and 20 µL of magnetic beads were introduced by pipetting. In this step, the negatively charged target

DNA bind to the positively charged magnetic beads (pH 5.0). In step 3, the DNA-carrying magnetic beads were enriched by a permanent magnet and the remaining supernatant was removed. Then 500 µL of washing buffer was introduced to remove possible inhibitors. In step 4, 150 µL of elution buffer was used to unbind the DNA from the magnetic beads due to charge repulsion (pH 8.8). In step 5, 1 µL of purified DNA was introduced to the LAMP master mix, which was transferred to the reagent compact disc and was run on the AnyMDx instrument for real-time amplification. All amplification curves of infected RBC samples showed clear exponential DNA amplification between 25 to 35 minutes (**Supplementary Figure 4B**). This result confirms the success of the magnetic bead-based method for malaria DNA extraction and purification.

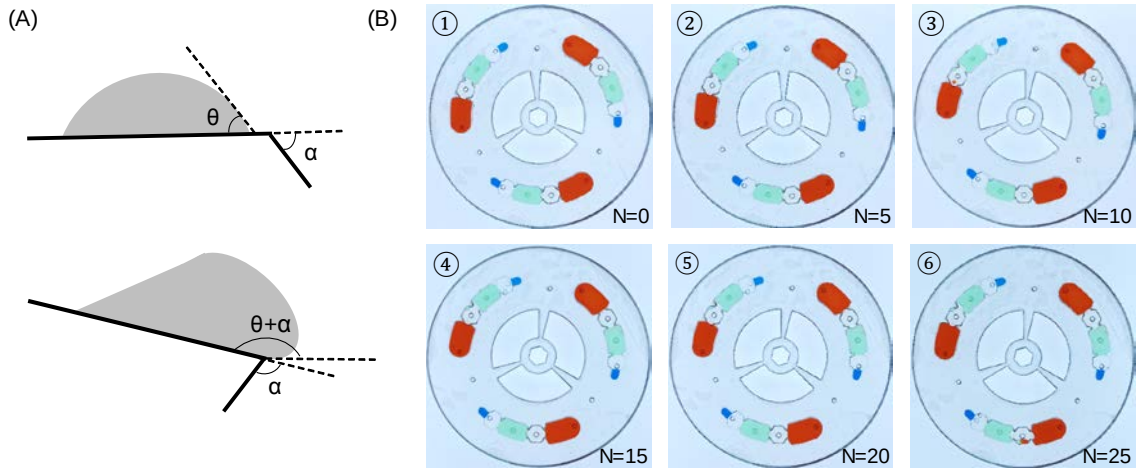
Supplementary Figure



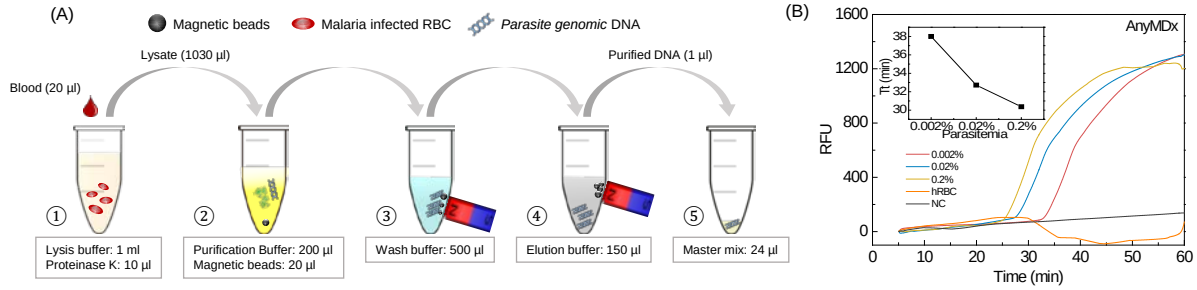
Supplementary Figure 1. The feedback-controlled reaction temperature profiles as a function of time. (blue curve: AnyMDx 1, red curve: AnyMDx 2)



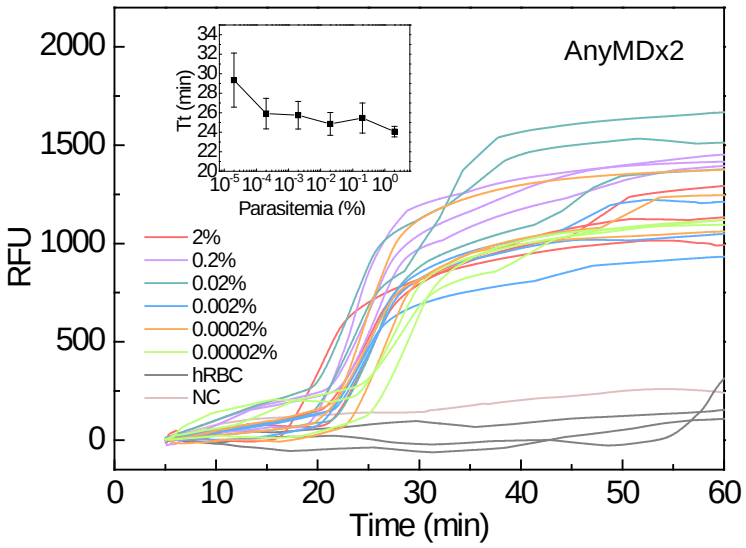
Supplementary Figure 2. Determination of the amplification threshold time (T_t). (A) A real-time amplification curve. (B) The differential profile of the real-time amplification curve (d*RFU*/d*t*), the max of which is used to determine the amplification threshold time. (T_t: threshold time, t: time, M_{max}: maximum value of the slope)



Supplementary Figure 3. Illustration of the pinning effect and photo images of the drop test results. (A) A droplet on a solid surface with a contact angle of θ, which will be increased up to $\theta+\alpha$ when moving towards a three-phase edge, where α is a bending angle[1]. This implies that larger α allows a higher activation barrier for the passive valve. (B) The drop test to evaluate the robustness of the teeth-shaped passive valves on the reagent compact disc under the harsh mechanical vibration. (N denotes the number of drops)



Supplementary Figure 4. (A) Illustration of the manual parasite genomic DNA extraction and purification procedures in a microcentrifuge tube. (B) The amplification curve for the manually extracted DNA sample on the AnyMDx instrument. The successful amplification of the tube-extracted DNA samples validates the effectiveness of the magnetic bead-based method.



Supplementary Figure 5. DNA amplification profiles of AnyMDx2. The manufacturing of the AnyMDx instrument is repeatable in a cost-effective way. We built the second instrument of AnyMDx (named AnyMDx2) and performed the similar sensitivity experiment (as described in sensitivity section). Amplification curves from six different parasitemia samples show clear exponential increases of fluorescence, while that of the negative controls (master mix and hRBC) shows no amplification. (RFU: relative fluorescence unit, hRBC: healthy RBCs, NC: negative control)

Supplementary Table

Supplementary Table 1. *P. falciparum* specific primer set

Primers	Sequences (5' → 3')
F3	CTC CAT GTC GTC TCA TCG C
B3	AAC ATT TTT TAG TCC CAT GCT AA
FIP (F1c-F2)	ACC CAG TAT ATT GAT ATT GCG TGA CAG CCT TGC AAT AAA TAA TAT CTA GC
BIP (B1-B2c)	AAC TCC AGG CGT TAA CCT GTA ATG ATC TTT ACG TTA AGG GC
LF	CGG TGT GTA CAA GGC AAC AA
LB	GTT GAG ATG GAA ACA GCC GG

Supplementary Table 2. Cost breakdown for AnyMDx instrument

System	Vendor	Description	Part#	Function	Unit Cost ($)	Unit Qty.	Ext Cost ($)
Bluetooth	Adafruit	Bluetooth Low Energy (BLE 4.0)	1697	Bluetooth	19.95	1	19.95
Electronics	Adafruit	Arduino Mega 2560 R3	DEV-1106	Microcontroller	45.95	1	45.95
Electronics	Adafruit	36-pin stripe male header	392	Headpins	4.95	0.083	0.41
Electronics	Newark	Detector Switch	83T2715	Switch	0.27	1	0.27
Electronics	Sparkfun	DC Barrel Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	Newark	Through Hole Resistor, 10 kΩ	38K0328	Temperature control	0.09	3	0.26
Electronics	Adafruit	Shield Stacking Headers for Arduino	85	Wire Sockets	1.95	0.33	0.64
Electronics	Newark	Through Hole Resistor, 100 Ω	38K0326	Resistors for LED	0.09	2	0.18
Electronics	Adafruit	Premium Male/Male Jumper Wires	758	Wires	3.95	0.75	2.96
Electronics	Newark	Diode, Standard, 1A, 50A	78K2043	Diode	0.07	1	0.07
Electronics	Newark	Trimmer Potentiometer, 10kΩ	16F7158	LCD adjustment	1.65	1	1.65
Enclosure	Mcmaster	Adjustable-Friction Hinge	1791A44	Hinge	6.72	2	13.44
Enclosure	Amazon	FLASHFORGE ABS Filament	90003001	3D platform material	18.50	0.3	5.55
Enclosure	Sparkfun	Metric Pan Head Machine Screws	92005A033	For holding LCD	3.67	0.04	0.15
Enclosure	Mcmaster	Acrylic Sheet, 1/8" Thick, 12" x 24"	8505K12	Holding plates	13.46	0.375	5.05
Enclosure	Thorlabs	Screws (M4 cap screw)	W8S038	For holding hinge	3.25	0.04	0.13
Enclosure	Thorlabs	Screws (M3 set screw)	SS3M6	For holding color sensor	9.25	0.0006	0.01
Enclosure	Home Depot	Screw Assortment Kit	800934	Hold plates	6.50	0.034	0.22
LCD	Sparkfun	Basic 16x2 Character LCD	LCD-00709	LCD	15.95	1	15.95
Magnets	Mcmaster	Neodymium Disc Magnet Nickel	58605K33	Holding magnetic beads	2.69	1	2.69
Optics	Adafruit	Color Sensor	1334	Detection	7.95	1	7.95
Optics	Edmund	Optical Plastic Light Guide2	#02-538	Guiding light	2.55	0.06	0.15
Optics	Newark	CREE LED, Blue, T-1 3/4 (5mm)	04R6674	Fluorescence excitation	0.21	1	0.21
Optics	Mcmaster	Dispensing Needle	75165A551	Holding optic fiber	13.00	0.02	0.26
Servo	Adafruit	Standard Size - High Torque Servo	1142	Actuation of disc	19.95	1	19.95
Thermal	Thermoelectric	Cold Plate	CP-0.91-0.91	Heating Stage	5.75	1	5.75
Thermal	Digikey	Peltier Heater	102-1667-ND	Heater	16.00	1	16.00
Thermal	Newark	N Channel Power MOSFET	63J7707	Switch for Peltier heater	1.66	1	1.66
Thermal	Newark	Thermistor	95C0606	Temperature sensing	7.34	1	7.34
Thermal	Newark	Capacitor 470uF	65R3137	Power Stabilizing	0.11	1	0.11
				Total Cost			**$ 176.16**

Supplementary Table 3. Disposable reagent compact disc cost per test

Reagents	Vendor	Function	Stock Vol (ml)	Unit Cost ($)	Volume (μL)/rxn	Ext Cost/rxn
NEB Isothermal Buffer	NEB	LAMP master mix	6	24.00	2.5	0.010
F3	IDT	LAMP master mix	1.4	9.22	0.25	0.002
B3	IDT	LAMP master mix	1.5	10.22	0.25	0.002
FIP	IDT	LAMP master mix	1.0	7.14	2.00	0.013
BIP	IDT	LAMP master mix	1.4	9.18	2.00	0.013
LF	IDT	LAMP master mix	1.7	11.86	1.00	0.007
LB	IDT	LAMP master mix	1.3	8.61	1.00	0.007
MgSO4	NEB	LAMP master mix	6	20.00	1.75	0.006
Calcein	Sigma-Aldrich	LAMP master mix	8000	133.00	0.63	0.000
MnCl2	Sigma-Aldrich	LAMP master mix	100	62.60	1.88	0.001
dNTP Mix	Thermo Fisher	LAMP master mix	3.2	107.00	3.50	0.117
Bst polymerase	NEB	LAMP master mix	1	264.00	1.00	0.264
UltraPure Water	VWR	LAMP master mix	20	91.88	7.25	0.033
Lysis Buffer	Invitrogen	Sample Prep.	800	142.00	1000.00	0.178
Purification Buffer	Invitrogen	Sample Prep.	20	28.97	30.00	0.043
Wash Buffer	Invitrogen	Sample Prep.	100	144.84	150.00	0.217
Proteinase K	Invitrogen	Sample Prep.	1	1.45	10.00	0.014
Magnetic Beads	Invitrogen	Sample Prep.	2	2.90	10.00	0.014
Acrylic Glue	ePlastics	Compact disc	118	9.69	1.5	0.041
1/32" Acrylic Sheet	ePlastics	Compact disc	-	14.98	-	0.098
1/16" Acrylic Sheet	ePlastics	Compact disc	-	17.72	-	0.058
					Total Cost	**$ 1.14**

Reference

1. L. C. Gao and T. J. McCarthy, *Langmuir*, 2006, **22**, 6234-6237.