ADVANCED MATERIALS TECHNOLOGIES

Supporting Information

for *Adv. Mater. Technol.,* DOI: 10.1002/admt.202101013

Point-of-Care Platform for Rapid Multiplexed Detection of SARS-CoV-2 Variants and Respiratory Pathogens

Alexander Y. Trick, Fan-En Chen, Liben Chen, Pei-Wei Lee, Alexander C. Hasnain, Heba H. Mostafa, Karen C. Carroll, and Tza-Huei Wang**

WILEY-VCH

Supporting Information

Point-of-care platform for rapid multiplexed detection of SARS-CoV-2 variants and respiratory pathogens

Alexander Y. Trick, Fan-En Chen, Liben Chen, Pei-Wei Lee, Alexander C. Hasnain, Heba H. Mostafa, Karen C. Carroll, Tza-Huei Wang**

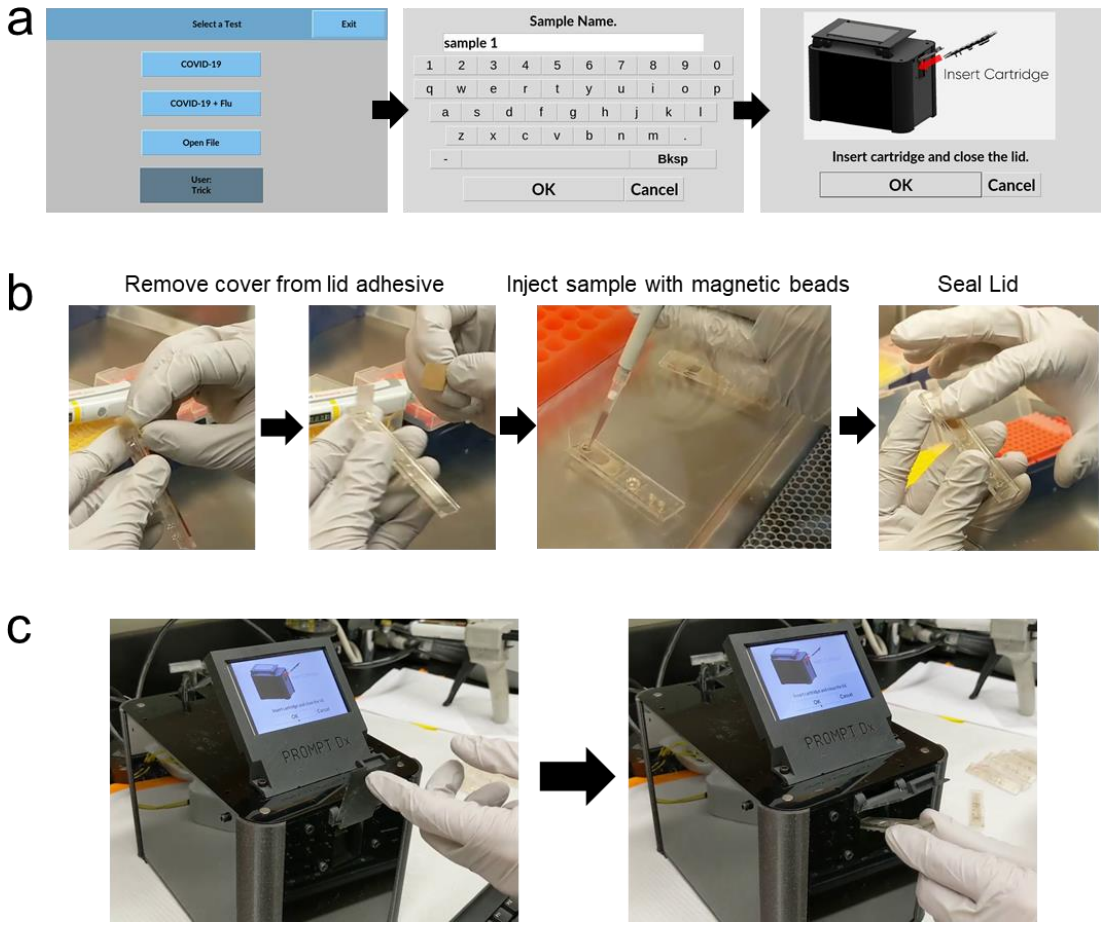

Figure S1. Loading cartridges and instrument operation

(**a**) Graphic user interface for assay selection, entry of sample name, and confirmation of cartridge insertion into the instrument. (**b**) To load the cartridge, first the paper cover is removed from the lid to expose a pressure-sensitive adhesive. Then the sample is mixed with magnetic beads and pipetted into the cartridge port followed by sealing the cartridge port with the adhesive lid. (**c**) The cartridge is inserted into the instrument slot PCR wells first. Once the user presses "OK" on the touchscreen, the instrument verifies the proper positioning of the cartridge wells, mounts the heat blocks, and initiates sample preparation and RT-PCR.

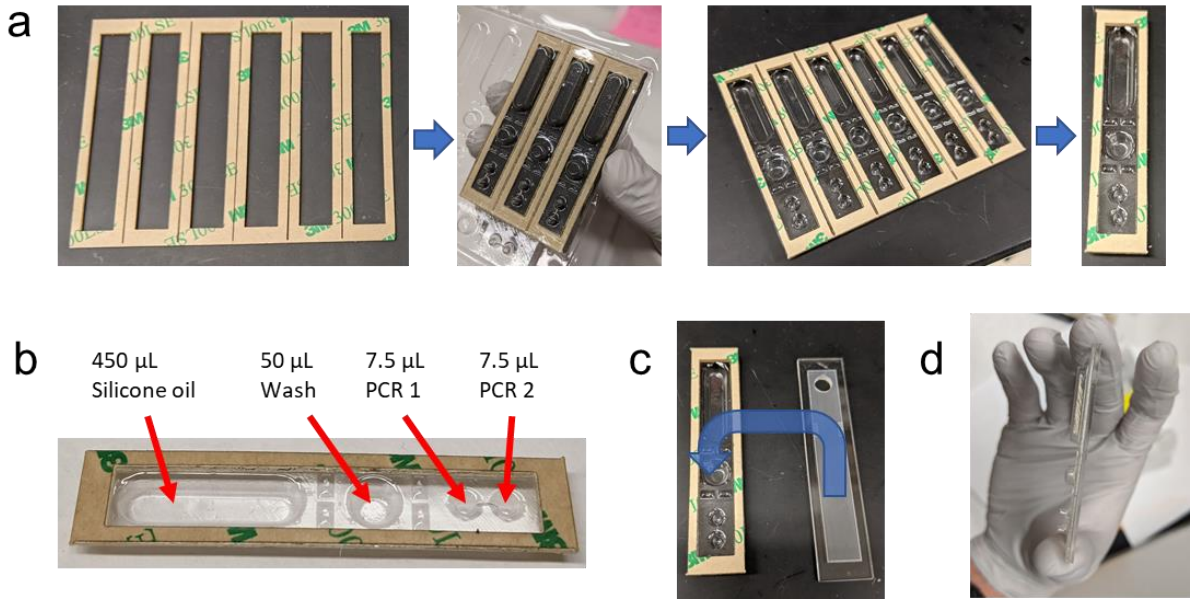

Figure S2. Cartridge assembly

(**a**) The middle section of the cartridge is a laser-cut spacer acrylic with pressure sensitive adhesive laminated to both sides. One side of the adhesive cover is removed and 3D-printed alignment mold presses the spacer into the thermoformed wells. Each thermoform contains a row of 6 cartridges which are individually cut out prior to reagent loading. (**b**) With the spacer section in place, silicone oil, wash buffer, and PCR buffers are dispensed directly into the thermoformed wells. (**c**) A top layer with laser-patterned Teflon tape and a laser-cut sample port seals the cartridge using the top layer of pressure sensitive adhesive on the spacer. (**d**) The cartridge is tilted to allow silicone oil to flow over the other reagent wells followed by dispensing of molten wax to plug the oil.

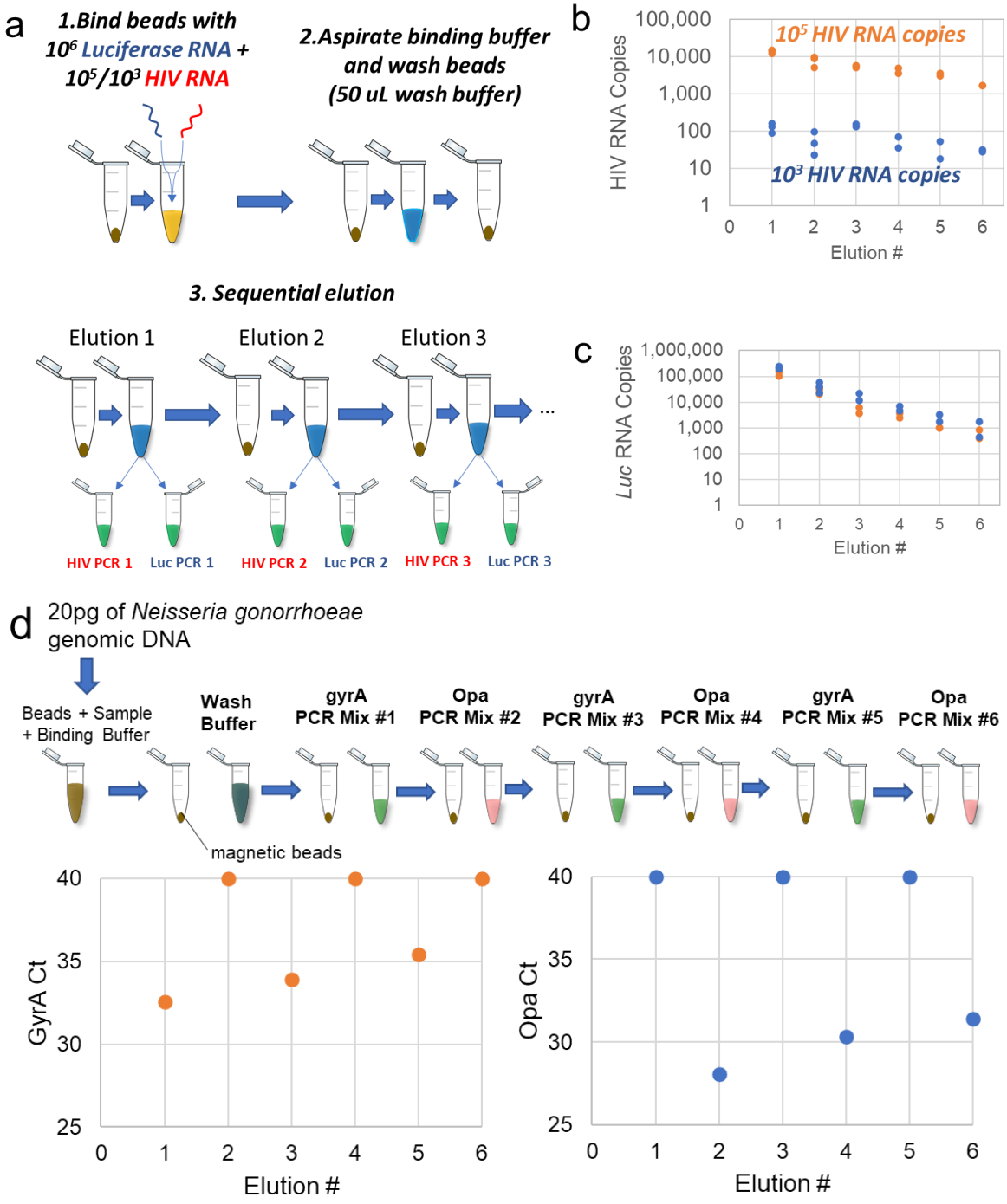

Figure S3. Nucleic acid aliquoting with sequential elution.

(**a**) To evaluate higher levels of potential multiplexing through sequential elution, 4 µL ChargeSwitch beads were mixed with 10 µL ChargeSwitch binding buffer and 20 µL target containing 10^6 copies of synthetic luciferase control RNA (L4561, Promega) with either 10^5 or 10^3 copies of synthetic HIV RNA (VR-3245SD ATCC). Beads were magnetically pelleted and the supernatant was aspirated and replaced with 50 µL ChargeSwitch wash buffer. After washing the beads, they were sequentially pelleted and resuspended into 10 µL ChargeSwitch elution buffer for a total of 6 elution steps. From each eluate, 1 µL was mixed into PCR assays for either amplification of HIV or luciferase (Luc) RNA. These assays contained 1X lyo-ready qPCR mix (Meridian Bioscience), 0.25 U PrimeScript RT enzyme (Takara Bio), 0.3

µM forward and reverse primers, and 0.25 µM probe (Table S4). Conditions for the first two elution steps were run in triplicate with duplicates for elution steps 3-6. (**b**) HIV RNA recovery was calculated for each of the 6 elution steps for tubes containing both 10^5 and 10^3 copies as the initial input. HIV RNA was successfully detected in all eluates with a steady decreasing trend as the number of elutions increased. (**c**) Luciferase RNA was also detected for all conditions with a trend that showed little deviation with either starting concentration of HIV target**.** (d) *Neisseria gonorrhoeae* genomic DNA and two *Neisseria gonorrhoeae* PCR assays targeting its *gyrA* gene and *Opa* gene were used to assess potential cross-contamination between each step of the sequential elution. The sequential elution was performed six times with alternating two PCR assays. No amplifications (Ct=40) were observed at the 2nd, 4th, and 6th elution steps for *GyrA*, or the 1st, 3rd, and 5th elution steps for *Opa*, indicating no measurable contamination caused by carry-over primer/probes during the sequential elution.

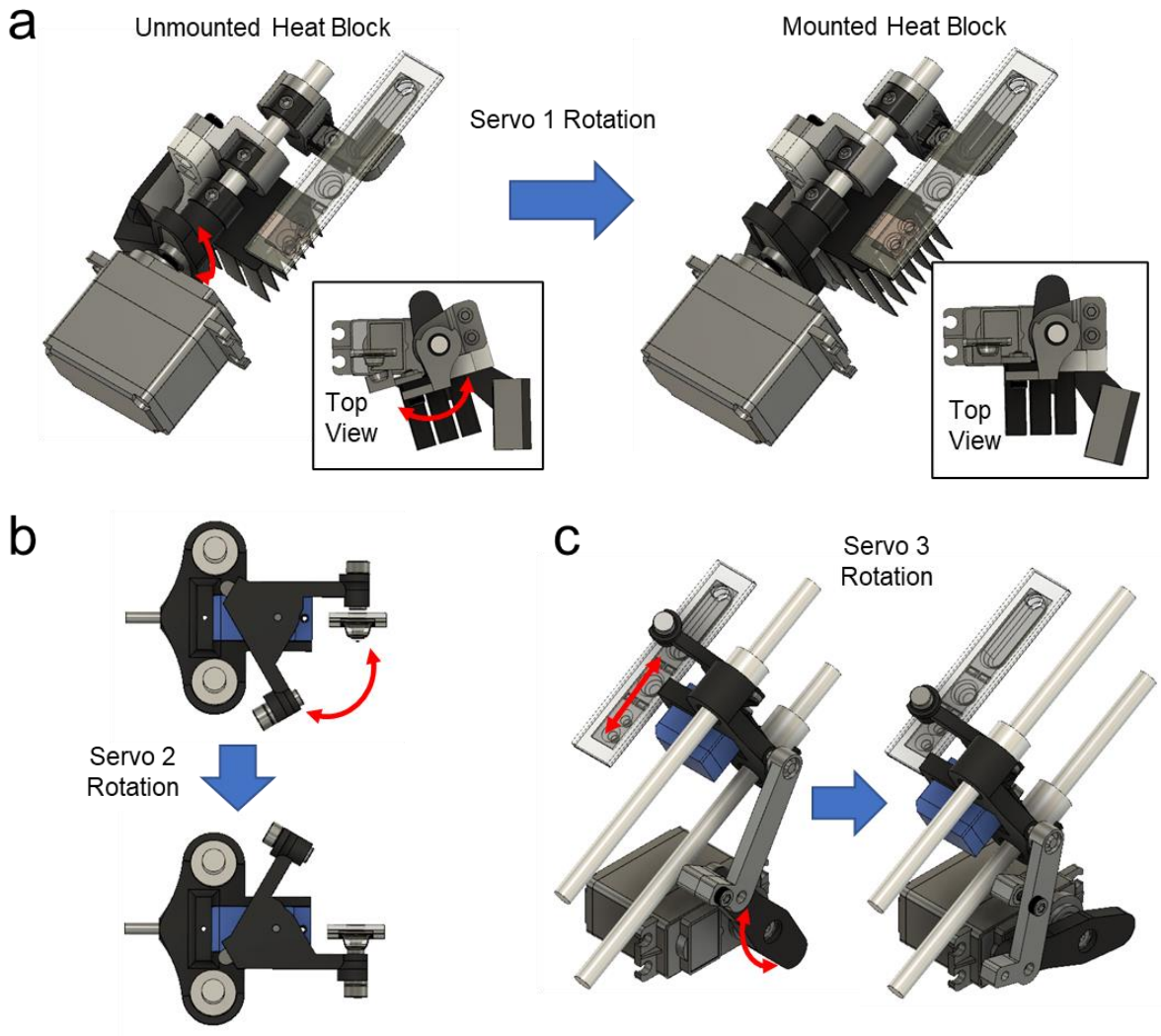

Figure S4. Servo actuation for cartridge mounting and magnetic transfer.
(**a**) After insertion of the cartridge, the first servo rotates to mount the heat blocks onto the PCR and sample wells. (**b**) Rotation of the second servo allows for transfer of magnetic beads into and out of cartridge wells. Here the rotation is shown pulling the beads from the planar PTFE-coated inner surface of the cartridge down into the reagent well. (**c**) The third servo is connected to lever arms that actuate the second servo along a linear axis while the beads are captured on the PTFE inner surface to transfer the beads between wells.

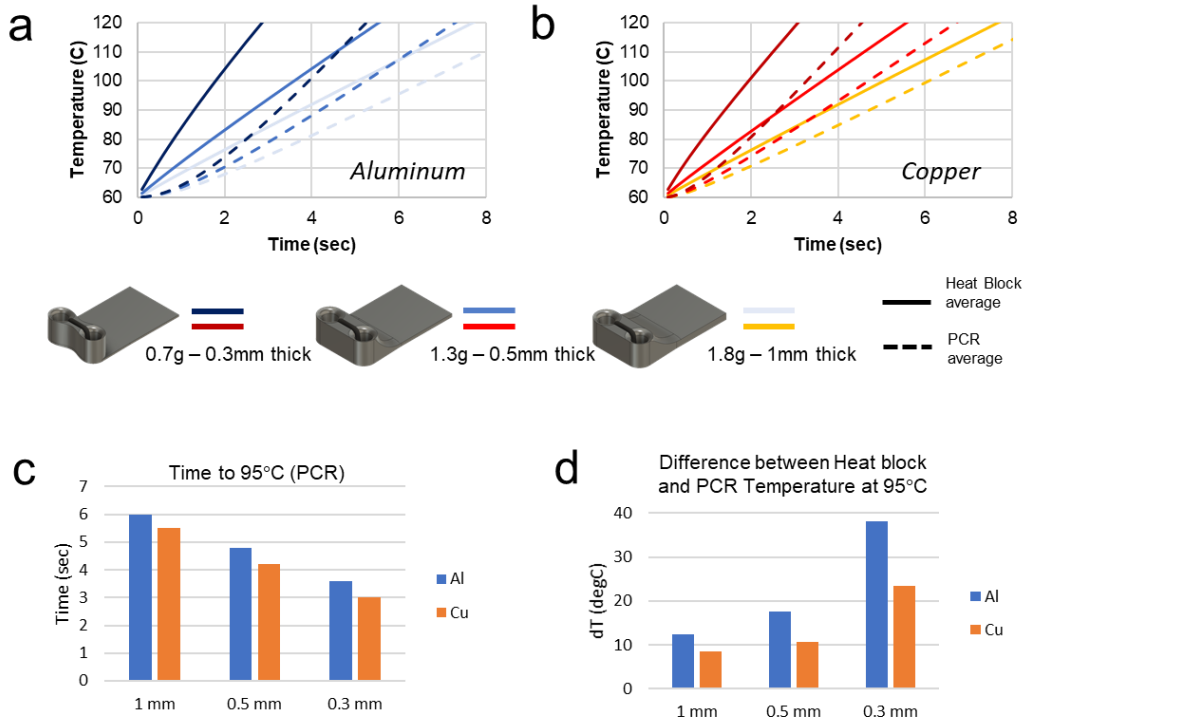

Figure S5. Heat block simulation and design for rapid thermocycling.

Transient heat transfer simulations were conducted in Solidworks 2019 using 3 different heat block models made from aluminum (**a**) or copper (**b**) with varying thickness. 7W constant heat power was supplied to the heat block in the rectangular section where the thermoelectric element would make contact. Average temperature for the heat block (solid lines) and 10 µL volumes of "PCR" (water) within each well (dashed lines) was tracked over 8 seconds. Reducing the thickness of the heat block resulted in faster heating, but larger differences between the heat block and PCR temperature. Copper's better thermal conductivity reduced the gradient between heat block temperature and PCR compared to aluminum. (**c**) Comparison of time for the PCR to reach 95°C from 60°C starting temperature given the 7W constant heating in varying thickness copper (Cu) or aluminum (Al) heat blocks. (**d**) Temperature difference between heat block and PCR when PCR first reaches 95°C indicates copper reduces the temperature gradient and requires less time to transfer heat from the thermoelectric to the PCR.

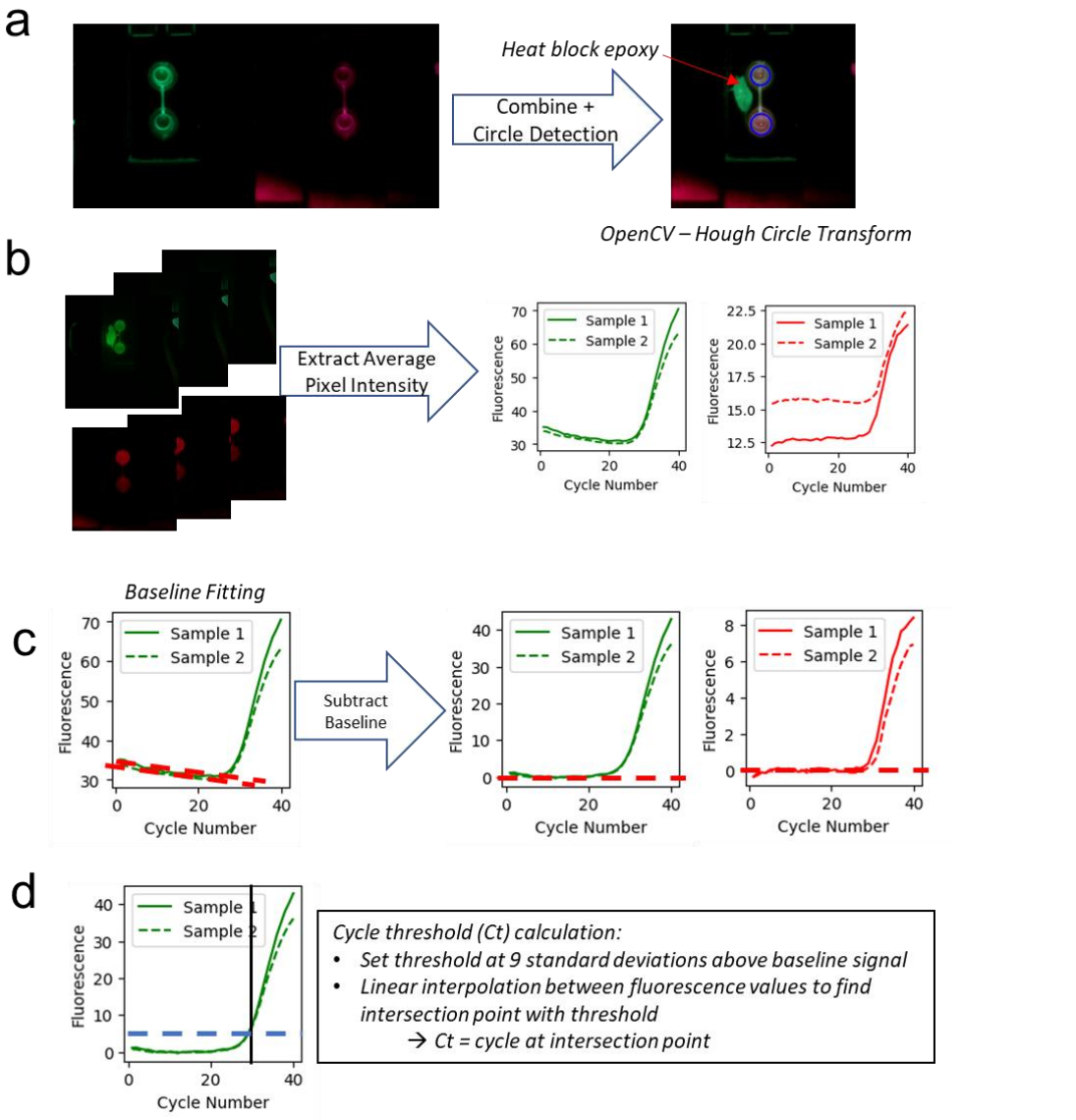

Figure S6. Fluorescence processing algorithm.

(**a**) The cartridge is first imaged upon insertion to verify its position using the python OpenCV Hough Transform to detect circles. Images are taken in both the red and green channels and combined. If the circles are within a threshold distance of the calibrated cartridge position, then the heat block is mounted (evident by the fluorescent epoxy used to hold the thermistor to the heat block) and the circles are redetected after mounting. (**b**) The detected circles are used to mask the images and extract the fluorescence for each individual well by averaging the pixel intensity. This results in the raw RT-PCR fluorescence curves. (**c**) Fluorescence curves are baseline corrected by fitting the baseline with a linear regression curve and subtracting the resulting line from the whole set of points for each well and fluorescence channel. (**d**) The baseline subtracted data is then used to calculate the cycle threshold (Ct) values for each curve using 9 standard deviations above the average fluorescence of the baseline as the threshold and interpolating the cycle and fluorescence data to find the cycle for the intersection point.

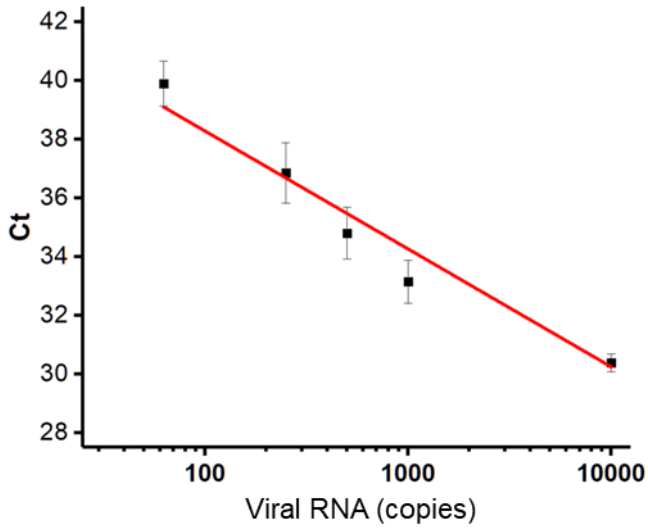

Figure S7. Detection of SARS-CoV-2 in saliva.

A serial dilution of known concentrations of gamma-irradiated viruses were spiked in pooled passive drool saliva samples (n = 4) collected from healthy donors. Five µL of each spiked saliva sample was mixed with 50 µL of magnetic bead buffer supplemented with thermo-labile proteinase K. The entire sample mix was then loaded into the cartridge for detection. The limit of detection was determined as 62.5 copies per 5 µL saliva input or 12.5 copies/µL, the lowest concentration of which at least three replicates were detected positive.

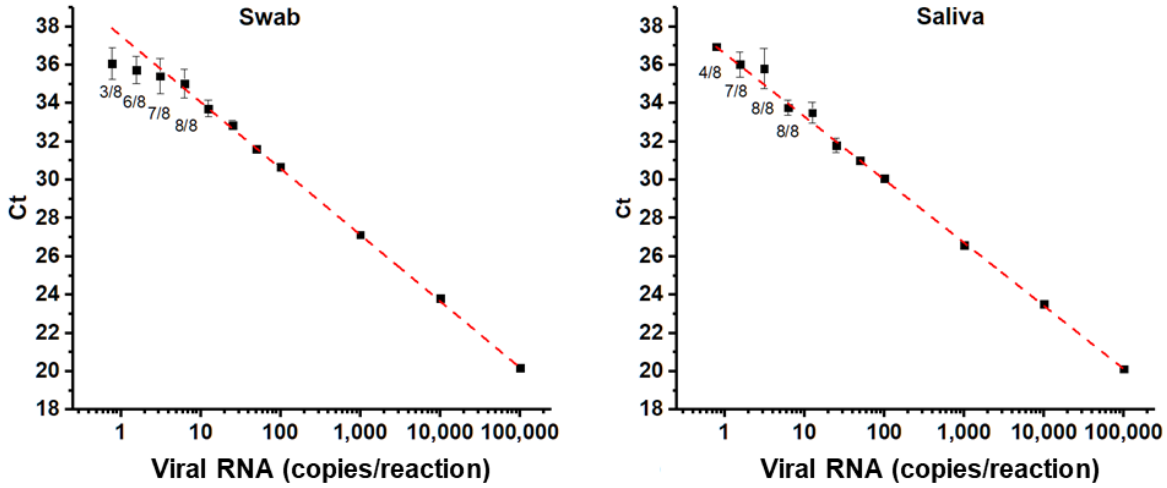

Figure S8. Comparator assay evaluation

Using the modified CDC protocol, we were able to detect 3 copies/µL of gamma-irradiated SARS-CoVID-2 viruses in swab eluate and 1.5 copies/µL in saliva. The labels in the graphs indicate detected positive rate of eight replicates at each concentration. We defined the limit of detection as positive results for at least 7 out of 8 repeats. Higher concentrations without number labels all tested positive (4 replicates). Red dashed lines represent linear regression.

Table S1. Cartridge bill of materials

Component	Vendor	Amount	Units	Price/Unit	Total Price
SpeedSTAR DNA Polymerase	Takara Bio	2.00	U	0.89	1.78
AccuStart II Taq	QuantaBio	2.00	U	0.23	0.45
QuantaBio ToughMix	QuantaBio	2.00	reaction	0.34	0.68
Oligonucleotides (Primers)	IDT	0.50	µL of 100 µM stock	0.06	0.03
Oligonucleotides (Probes)	IDT	0.20	µL of 100 µM	0.80	0.16
Top Acrylic Sheet	Eplastics	0.00	sheet	22.40	0.03
Middle Spacer Acrylic	McMaster-Carr	0.01	sheet	5.49	0.05
Thermoformed Wells	Welch Fluorocarbon	0.04	sheet	4.00	0.14
Silicone Oil	Thermofisher	0.42	mL	0.37	0.16
Chargeswitch Kit (Wash,Bind, Beads)	Thermofisher	4.00	µL beads	0.13	0.53
Teflon Tape	McMaster-Carr	0.05	foot	2.11	0.11
9472LE 3M Transfer Tape	Uline	0.07	foot	1.46	0.10
DEPC Water	N/A	3	µL	negligible	0.00
Tween-20	Sigma-Aldrich	0.02	µL	negligible	0.00
				Price per cartridge	**4.22**

Table S2. Instrument bill of materials

Category	Part Description	Vendor	Model	Qty	Unit Price	Cost
Housing	M3 Screws	Amazon	DYWISHKEY 360 Pieces	0.5	$11.69	$5.85
Optics	Raspberry Picamera	Amazon	NoIR V2 8MP	1	$23.99	$23.99
Electronlcs	Arduino Nano (Elegoo)	Amazon	ELEGOO Nano pack of 3	0.333	$19.98	$6.65
Electronlcs	Touchscreen	Amazon	ELECROW 800x480 Touch Screen	1	$49.99	$49.99
Electronlcs	HDMI Cables	Amazon	Cable Matters 3-pack	0.33	$12.99	$4.33
Electronlcs	USB Cables	Amazon	Sabrent 1ft - pack of 6	0.16	$7.98	$1.33
Electronlcs	Power Supply Cord	Amazon	6'	1	$7.42	$7.42
Electronlcs	24 AWG stranded wire	Amazon	Silicone wire 24awg	0.01	$16.99	$0.17
Motors	Linear Ball Bearing	Amazon	LM6UU - 12 pieces	0.25	$7.99	$2.00
Motors	uxcell Ball Bearing	Amazon	ABEC-3	1	$8.89	$8.89
Electronlcs	Raspberry Pi 3B+	Canakit	3B+	1	$35.00	$35.00
Optics	Excitation Filter	Chroma	59003m	1	$350	$350.00
Electronlcs	MCP3008	Digikey	MCP3008-I/P	1	$2.19	$2.19
Motors	Sub Micro Servo	Digikey	SG51R	1	$5.95	$5.95
Temperature Control	5 Ohm Power Resistor	Digikey	PF1262-5RF1	1	$2.64	$2.64
Housing	3D-printed Fixtures	Formlabs	Black Resin 1L	0.2	$149	$29.80
Electronlcs	Custom PCB	JLCPCB	Y6-2808702A	0.2	$19.55	$3.91
Magnets	Magnets - 3/16" x 1/4"	K&J Magnetics	D34-N52	2	$0.47	$0.94
Magnets	Magnets - 1/4" x 1/8"	K&J Magnetics	D42-N52	6	$0.43	$2.58
Electronlcs	BuckPuck	LuxeonStarLEDs	3021-D-E-350	2	$9.02	$18.04
Temperature Control	25 mm heatsink	LuxeonStarLEDs	25x25mm	1	$5.34	$5.34
Housing	Black Cast Acrylic Sheet	McMaster	12"x24"x1/8"	1	$14.27	$14.27
Motors	1/8" Aluminum Rod	McMaster	3ft	0.2	$10.88	$2.18
Motors	6 mm Aluminum Rod	McMaster	3ft	0.333	$24.59	$8.19
Motors	6mm set screw collar	McMaster		3	$2.11	$6.33
Temperature Control	Easy-to-Machine 145 Copper Bar	McMaster	1/4"x2"x1ft	0.04	$38.43	$1.60
Electronlcs	Power Supply 7.5V 6A	Mouser	MEAN WELL GST60A07-P1J	1	$19.30	$19.30
Temperature Control	100kOhm Thermistor	Mouser	GA100K6MCD1	2	$19.00	$38.00
Optics	Emission Filter	Omega	535-700DBEM	1	$300	$300.00
Electronlcs	Dual TB9051FTG Motor Driver for Raspberry Pi	Pololu	2761	1	$21.95	$21.95
Electronlcs	5V 5A Voltage Regulator	Pololu	2851	1	$14.95	$14.95
Motors	HS485HB Servo motors	RobotShop	HS-485HB	2	$17.99	$35.98
Optics	Vollong 3W RGB High Power LED	SuperBrightLEDs	VL-H01RGB00302	1	$4.95	$4.95
Temperature Control	TEC	Custom Thermoelectric	02301-9B30-32RU6A	1	$42.20	$42.20
					Total Cost:	$1,076.90

Table S3. Specificity testing

ND = no false-positive amplification detected

Organism	Source	Concentration	Ct Value
Human coronavirus 229E	Bei Resources NR-52726	3.64×10^7 genome equivalents/mL	ND
Human coronavirus NL63	Bei Resources NR-470	3.34×10^7 genome equivalents/mL	ND
Respiratory syncytial virus	Bei Resources NR-4052	136 ng/mL	ND
Human metapneumovirus	Bei Resources NR-22227	258 ng/mL	ND
Acinetobacter baumannii	ATCC 19606	10^9 CFU/mL	ND
Enterococcus faecalis	ATCC 29212	10^9 CFU/mL	ND
Enterococcus faecium	ATCC 35667	10^9 CFU/mL	ND
Escherichia coli	ATCC 25922	10^9 CFU/mL	ND
Klebsiella pneumoniae	ATCC BAA-1705	10^9 CFU/mL	ND
Morganella morganii	ATCC 25830	10^9 CFU/mL	ND
Mycobacterium tuberculosis	ATCC 25177	10^9 CFU/mL	ND
Staphylococcus aureus	ATCC 29213	10^9 CFU/mL	ND
Streptococcus agalactiae	ATCC 13813	10^9 CFU/mL	ND
Pseudomonas aeruginosa	ATCC 27853	10^9 CFU/mL	ND

Table S4. PCR assay primers and probes

Set name	Primer/probe	Sequence (5' → 3')	Reference
SARS-CoV-2 N1	Forward primer	GACCCCAAAATCAGCGAAAT	*27*
	Reverse primer	TCTGGTTACTGCCAGTTGAATCTG	
	Probe	/FAM/ACCCCGCAT/ZEN/TACGTTTGGTGGACC/IABkF	
Control RNA	Forward primer	TACAACACCCCAACATCTTCGA	*26*
	Reverse primer	GGAAGTTCACCGGCGTCAT	
	Probe	/5TYE665/CGGGCGTGGCAGGTCTTCCC/3IAbRQSp/	
Influenza A	Forward primer	CTTCTAACCGAGGTCGAAACGTA	*29*
	Reverse primer	GGTGACAGGATTGGTCTTGTCTTTA	
	Probe	/5TYE665/TCAGGCCCCCTCAAAGCCGAG/3IAbRQSp/	
Influenza B	Forward primer	AAATACGGTGGATTAAACAAAAGCAA	*28*
	Reverse primer	CCAGCAATAGCTCCGAAGAAA	
	Probe	/56-FAM/CACCCATATTGGGCAATTTCCTATGGC/3IABkFQ	
Yale ORF1a Δ3675-3677	Forward primer	TGCCTGCTAGTTGGGTGATG	*10*
	Reverse primer	TGCTGTCATAAGGATTAGTAACACT	
	Probe	/5Cy5/GTTTGTCTG/TAO/GTTTTAAGCTAAAAGACTGTG/3IAbRQSp	
Yale Spike Δ69-70	Forward primer	TCAACTCAGGACTTGTTCTTACCT	
	Reverse primer	TGGTAGGACAGGGTTATCAAAC	
	Probe	/56-FAM/TTCCATGCT/ZEN/ATACATGTCTCTGGGA/3IABkFQ/	
HIV	Forward primer	CATGTTTTCAGCATTATCAGAAGGA	*44*
	Reverse primer	TGCTTGATGTCCCCCCACT	
	Probe	/56-FAM/CCACCCCAC/ZEN/AAGATTTAAACACCATGCT AA/3IABkFQ/	

Movie S1. On-cartridge sample processing

Data S1. Clinical samples data (separate file)