Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Information

UbiNAAT: Multiplexed Point-of-Care Nucleic Acid Diagnostic Platform for Rapid At-Home Pathogen Detection

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Table S1. Nucleotide sequences of LAMP primers that target the pandemic influenza A gene. [62]

Primer	Sequence (5' – 3')	Final Concentration
F3-1	GACTTGAAGATGTCTTTGC	0.1 µM
F3-2	GACTGGAAAGTGTCTTTGC	0.1 µM
B3-1	TRTTATTTGGGTCTCCATT	0.1 µM
B3-2	TRTTGTTTGGGTCCCCATT	0.1 µM
FIP	TTAGTCAGAGGTGACARRATTGCAGATCTTGAGGCTCTC	1.6 µM
BIP	TTGTKTTCACGCTCACCGTGTTTGGACAAAGCGTCTACG	1.6 µM
Loop F	GTCTTGTCTTTAGCCA	0.4 µM
Loop B	CMAGTGAGCGAGGACTG	0.4 µM

Table S2. Nucleotide sequences of LAMP primers that target the SARS-CoV-2 N gene.[63]

Primer	Sequence (5' – 3')	Final Concentration
F3	TGGCTACTACCGAAGAGCT	0.2 µM
B3	TGCAGCATTGTTAGCAGGAT	0.2 µM
FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	1.6 µM
BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	1.6 µM
Loop F	GGACTGAGATCTTTCATTTTACCGT	0.4 µM
Loop B	ACTGAGGGAGCCTTGAATACA	0.4 µM

Table S3. Nucleotide sequences of LAMP primers that target the respiratory syncytial virus (RSV) gene.[65]

Primer	Sequence (5' – 3')	Final Concentration
F3	GCTGTTCAATACAATGTCCTAGA	0.2 µM
B3	GGTAAATTTGCTGGGCATT	0.2 µM
FIP	TCTGCTGGCATGGATGATTGGAGACGATGATCCTGCATCA	1.6 µM
BIP	CTAGTGAAACAAATATCCACACCCAGCACTGCACTTCTTGAGTT	1.6 µM
Loop F	ACATGGGCACCCATATTGTAAG	0.4 µM
Loop B	AGGGACCTTCATTAAGAGTCATGAT	0.4 µM

Table S4. Nucleotide sequences of LAMP primers that target the POP7 gene.[64]

Primer	Sequence (5' – 3')	Final Concentration
F3	TTGATGAGCTGGAGCCA	0.2 µM
B3	CACCCTCAATGCAGAGTC	0.2 µM
FIP	GTGTGACCCTGAAGACTCGGTTT-TAGCCACTGACTCGGATC	1.6 µM
BIP	CCTCCGTGATATGGCTCTTCGTTT-	1.6 µM

	TTTTCTTACATGGCTCTGGTC	
Loop F	ATGTGGATGGCTGAGTTGTT	0.4 µM
Loop B	CATGCTGAGTACTGGACCTC	0.4 µM

Table S5. In-tube and QMA-based LAMP master mix

Reagent	**Final Concentration (In-tube)**	**Final Concentration (QMA)**
WarmStart LAMP Kit	1X	1X
SYTO-82	7.5 µM	7.5 µM
Hydroxynaphthol Blue	40 µM	40 µM
Assay specific primers (See Tables S1-S4)	varies	varies
Trehalose	10%	10%
Dextran, 500 kD	0.5%	0.5%
Sample	1 µL in volume	1 µL in volume
Nuclease-Free H_2O	Fill up to 20 µL	Fill up to 25 µL

Table S6. Cost analysis of amplification assay reagents

	Component	**Manufacturer**	**Cost/Device**
RT-LAMP Reagents	WarmStart RNA/DNA LAMP Kit	New England Biolabs	$1.50
	SYTO-82	Thermo Fisher	<$0.01
	Hydroxynaphthol Blue	Millipore Sigma	<$0.01
	Trehalose	Life Sciences	<$0.01
	20X Primer Mix	Integrated DNA Technologies	$0.05
	Dextran, 500 kDa	Millipore Sigma	<$0.01
	TCEP	Millipore Sigma	<$0.01
	EDTA	Life Technologies	<$0.01
	Tris-HCl	Invitrogen	<$0.01
		Subtotal	***$1.55***

Table S7. Cost analysis of UbiNAAT device components

	Component	**Manufacturer**	**Cost/Device**
UbiNAAT Device	Poly methyl methacrylate (PMMA), 1/16”	McMaster-Carr	$0.25

(Non-Reuseable)	Poly methyl methacrylate (PMMA), 3s/8”	McMaster-Carr	$0.06
	Poly methyl methacrylate (PMMA), 1/8”	McMaster-Carr	$0.24
	Polydimethylsiloxane (PDMS) tape-silicone transfer	Valley Industrial Products	$0.90
	Glass fiber 8950	Ahlstrom	<$0.01
	Tyvek	Du Pont	<$0.01
	Dental Wax	Electron Microscopy Sciences	<$0.01
	Quartz microfiber (QMA)	Cytiva (VWR)	<$0.01
	3M™ Copper Conductive Tape	Ted Pella	<$0.01
	Aluminum Foil	Reynolds	<$0.01
	Thermal Tape	Newark	$0.05
	Aluminum Foil Tape (Black)	Advance Tapes	<$0.01
UbiNAAT Enclosure (Reusable)	White Resin	Formlabs	$18.00
	Poly methyl methacrylate (PMMA), 1/16”	McMaster-Carr	$0.10
	Mylar (0.004” thickness)	Fralock	<$0.01
Cell Phone Fluorescence Filters (Reuseable)	Emission filter FES0550 - Ø1" Shortpass Filter, Cut-Off Wavelength: 550 nm	Thorlabs	$1360
	Excitation filter BP 587/25 (HE)	Zeiss	$80
		Subtotal	***$1459.60***
		Subtotal (Reusable)	***$1458.10***

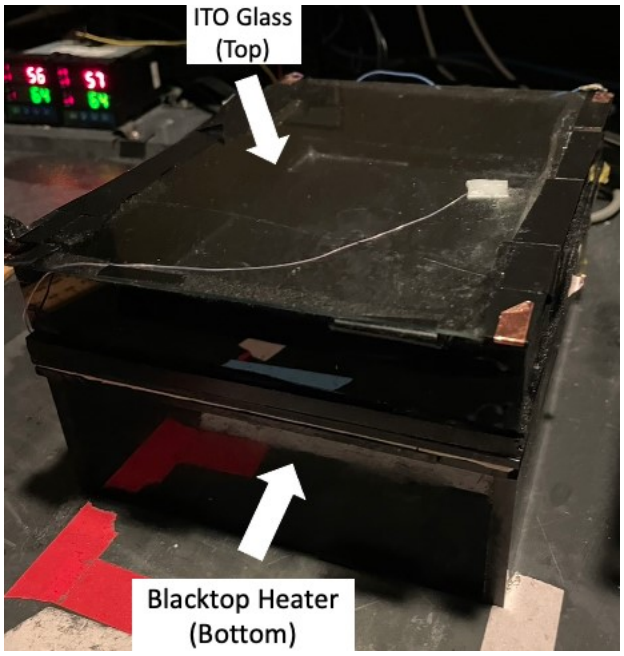


Figure S1. Custom dual-sided heater for testing of QMA-based RT-LAMP assays in 5-pad devices. Heater top (ITO glass) and blacktop bottom heaters are each connected to PID temperature controller.

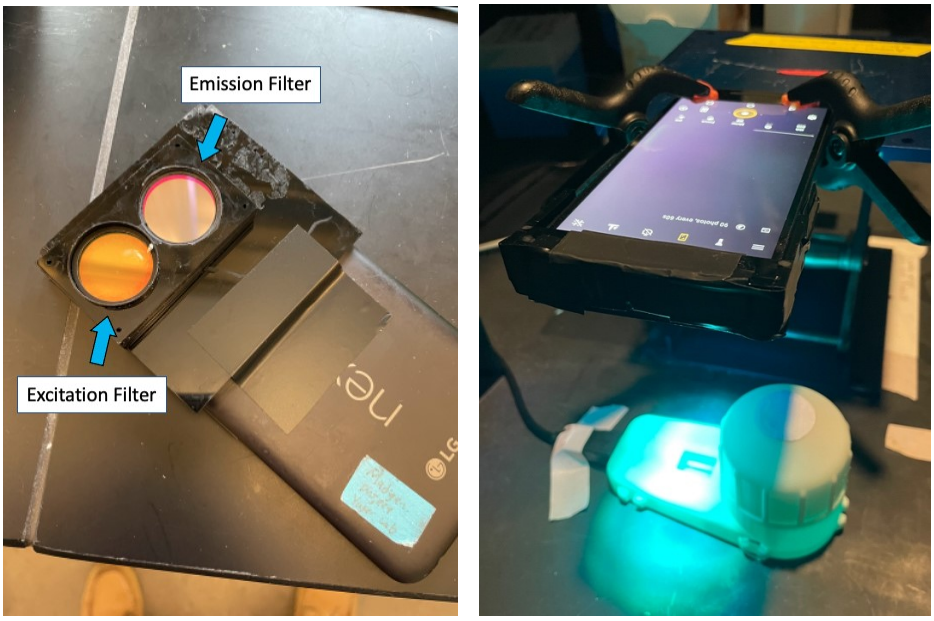


Figure S2. (*left*) Google Nexus 5 cell phone fitted with a custom PMMA fixture holding an excitation filter (Zeiss Bandpass 587/25 (HE)) and emission filter (Thorlabs Ø1 in. Shortpass Filter, Cut-Off Wavelength: 550 nm). (*right*) Cell phone reader fixed on stand 8.5 cm above device amplification pad region. Camera settings are: (1) Incandescent white balance (2) Manual focus with a focal length of 8.5 cm between lens and image target (3) 200 ISO (4) 1/5 second shutter speed (5) Time interval of 1 image per minute for 60 minutes

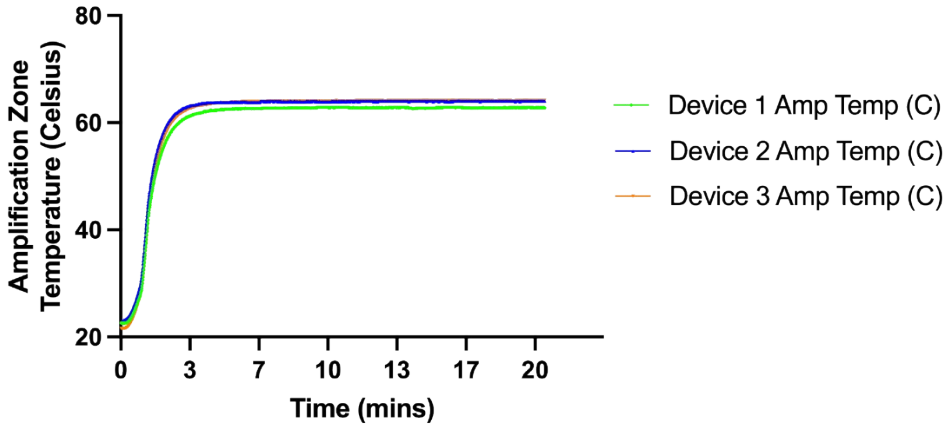


Figure S3. Validation of UbiNAAT QMA pad temperature being within target range (62-65°C) during amplification heating. In-pad temperature across three separate UbiNAAT devices collected via T-type thermocouple (Omega) inserted into amplification zone.

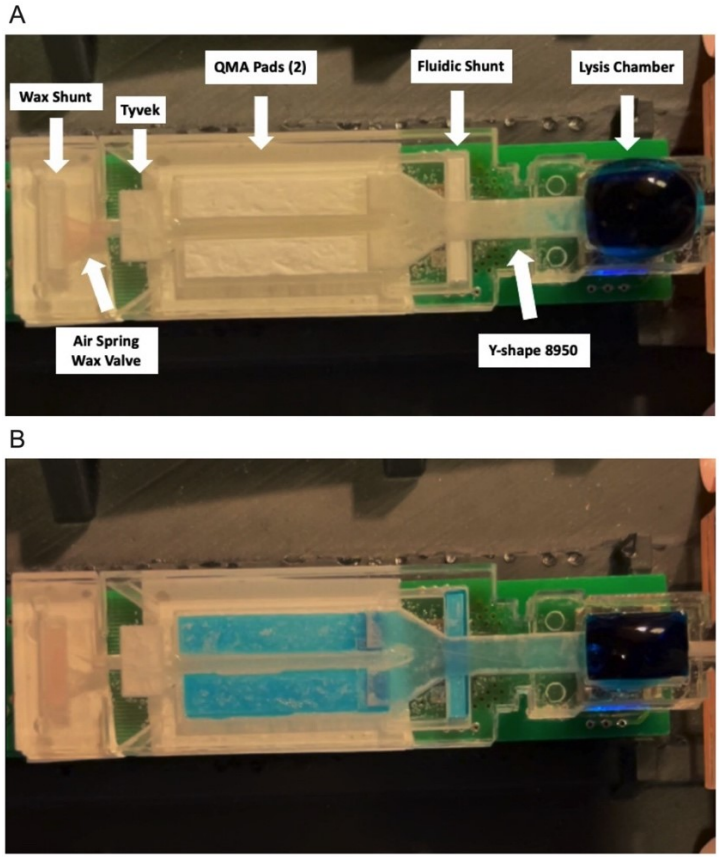
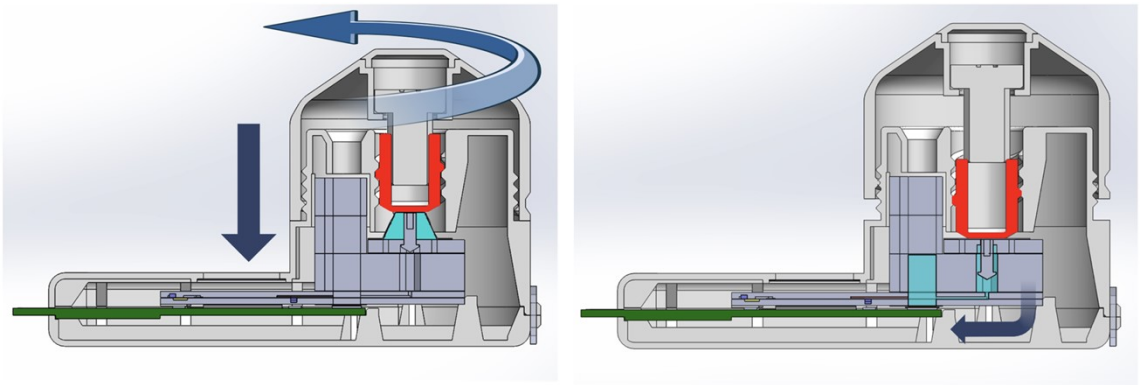


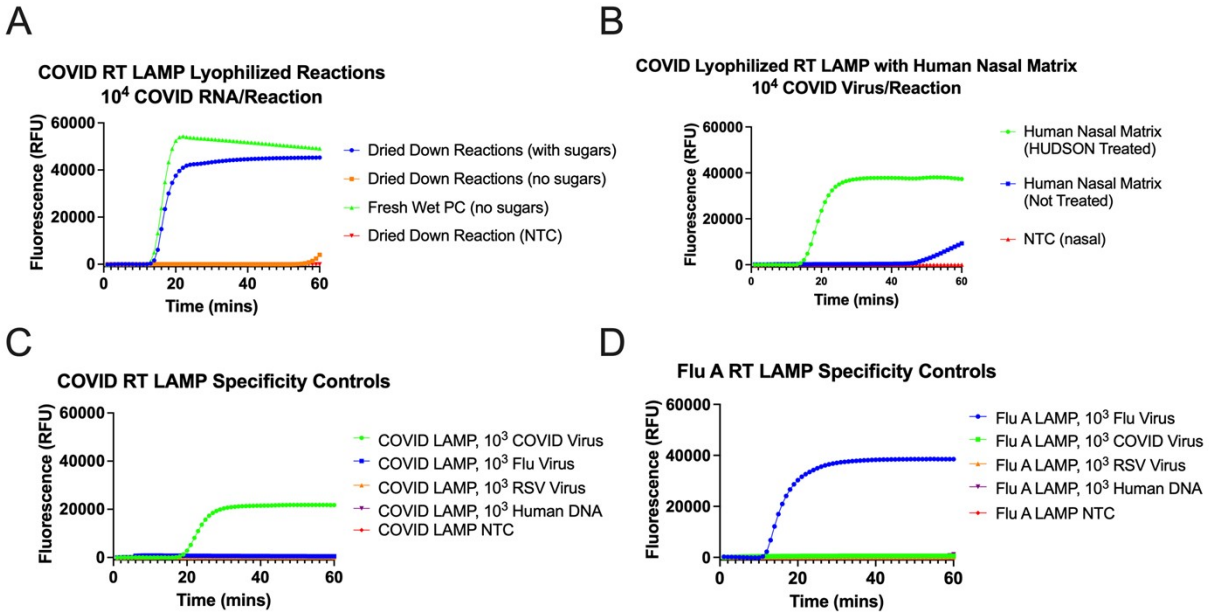
Figure S4. Fluidic flow path within UbiNAAT device, as controlled by the air spring terminal wax valve. Fluidic path is shown with food coloring in (**A**) labeled fluidic path from lysis chamber to QMA pads, and is enabled by PCB resistive heating of wax valve (**B**) fully resuspended device. Tyvek is added between the amplification pads and the wax valve to prevent lysate fluidic flow towards the air vents.



Step 1: As cap is removed by turning counter-clockwise, an internal activator pin (*red*) is permanently lowered to compress the blister pack (*light blue*)

Step 2: The compressed blister pack is punctured by an internal sharp, pushing buffer into the lysis chamber through the channel

Figure S5. Schematic of blister pack operation.



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	Viral Sample Detection (copy/reaction)	Liftoff Time, Average ± Std. Dev. (mins)
COVID-19	10000	13.5 ± 0.8
	100	18.5 ± 2.3
FLU A	10000	11.9 ± 0.7
	100	16.3 ± 2.1

Figure S6. In-tube RT-LAMP assay development for COVID-19 and Influenza A detection (*mean ± SD*) for (**A**) At 10^4 RNA copies per reaction, COVID lyophilized RT-LAMP reactions containing trehalose and dextran (13.7 ± 0.2 mins, n=3) show similar liftoff times to freshly prepared RT-LAMP reactions (12.3 ± 0.4 mins, n=3). Lyophilized reactions without sugars and non-template controls (NTC) showed no significant signal liftoff in 60 minutes of amplification. *Note that LAMP Fluorescent Dye (NEB) was used instead of SYTO-82 in Figure S6A.* (**B**) HUDSON-treated nasal matrix RT-LAMP reactions show early signal liftoff (14.6 ± 0.5 mins, n=3) while untreated nasal matrix reactions do not show reliable amplification across replicates (**C**) COVID RT-LAMP is specific against human DNA, Flu, and RSV viruses (n=1) (**D**) Flu A RT-LAMP is specific against human DNA, COVID, and RSV viruses (n=1) (**E**) Fluorescence signal liftoff times for lyophilized COVID and Flu A RT-LAMP assays showing pre-20 minute signal liftoff across viral copy range (n=3)

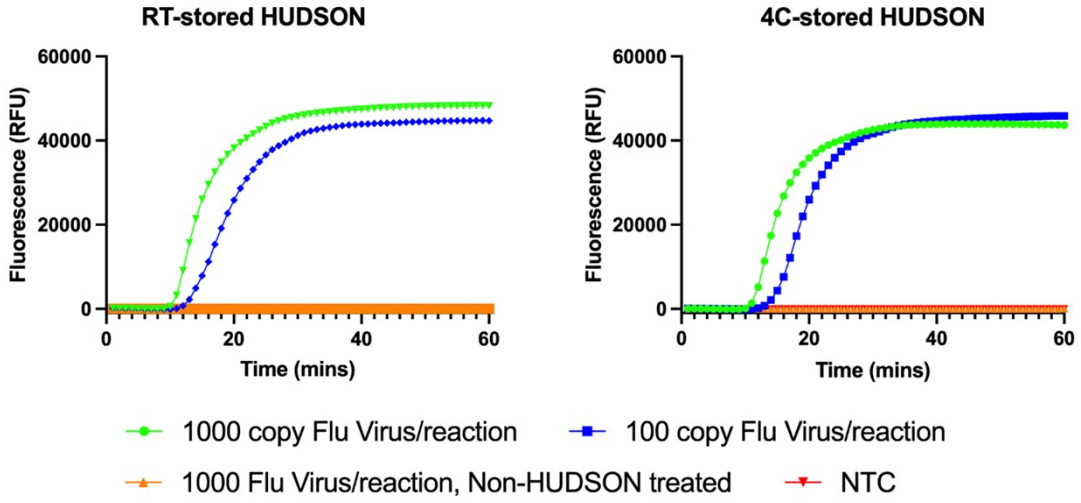

Figure S7. Flu A RT-LAMP indicating HUDSON solution stability for inactivation of human nasal matrix following 15 days of storage at either ambient temperature (RT) or 4°C refrigerator (averaged fluorescence signal across n=3 samples). Solutions were freshly prepared at 1X working concentration (2.5 mM TCEP, 1 mM EDTA, pH 8.0) prior to storage.

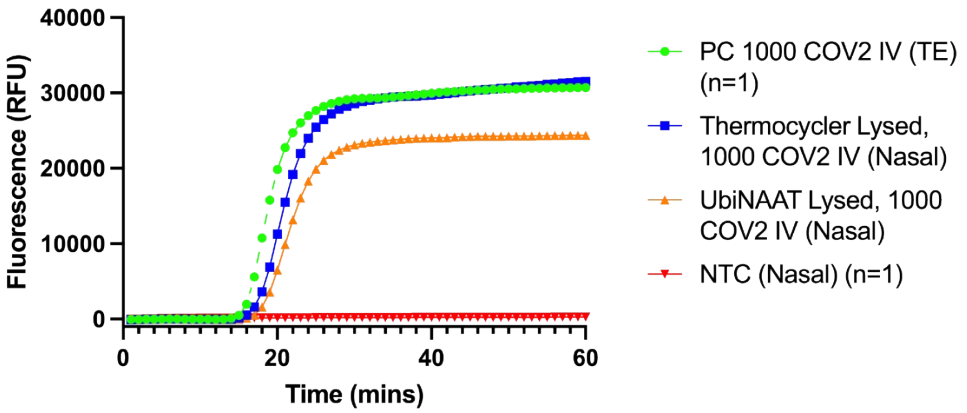

Figure S8. SARS-CoV-2 RT-LAMP evaluation of UbiNAAT lysis compared to thermocycler lysis (BioRAD MJ Mini 48-well) of 1000 copies of COVID inactivated virus (ATCC) (averaged fluorescence signal across n=3 replicates). BioRAD thermocycler lysis set to 95°C for 5 minutes, and UbiNAAT lysis set to 100% power for 7 minutes (100°C for 3 mins).

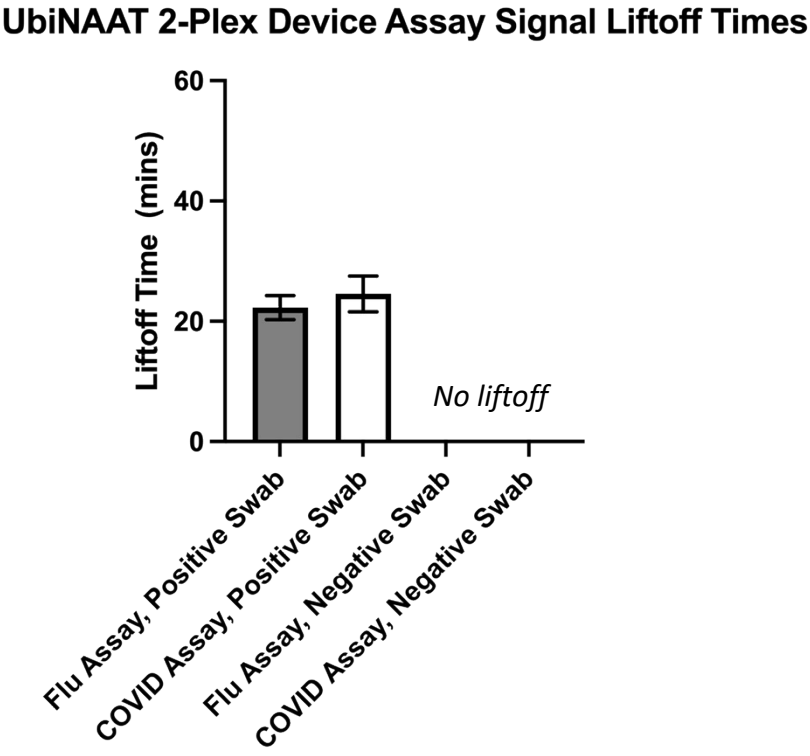

Figure S9. Liftoff times for positive and negative human nasal swab samples as processed in UbiNAAT device (*mean ± standard deviation*). Positive swab samples for COVID assay (24.6 ± 3.0 mins, n=5) and Flu A assay (22.3 ± 2.0 mins, n=5) both showed liftoff prior to 30 minutes, while

none of the COVID negative swab samples (n=3) nor the Flu A negative swab samples (n=3) showed signal liftoff within 60 minutes of amplification.