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Supplementary information

Rapid detection of SARS-CoV-2 RNA in saliva via Cas13

In the format provided by the authors and unedited

Contents

Supplementary Table 1: LAMP primer sets screened, Related to Figure 2

Lamp Primer Set	Time to Amplification (min)	NTC-Amplification time delta (min)	Amplicon length (nt)	Reference
Orf1ab Set 1	11.6	21.3	9	(Rabe and Cepko 2020)
N Set 2	12.2	28.7	26	(Joung et al., n.d.)
N Set 1	12.5	33.1	46	(Broughton et al. 2020)
Nsp3 Set 1	13.2	30.0	1	(Park et al. 2020)
Orf1ab Set 2	14.8	25.4	1	(Yu et al. 2020)
N Set 3	14.5	27.1	27	(Zhang et al. 2020)
N Set 4	15.7	25.4	27	(Zhang et al. 2020)

Orf1ab Set 3	16.7	21.5	0	(Lamb et al., n.d.)
E Set 1	18.0	27.6	60	(Broughton et al. 2020)

Supplementary Table 2: LAMP, rLAMP, qRT-PCR, and Cas13 sequences

See attached file

Supplementary Table 3: Fluidics calibration volumes

Volumes (µL)	**Saliva**	**Primer Mix**	**Master Mix**	**Cas13+crRNA**	**T7mix + FQ**
Loaded	150	12	40	10	40
Lost	-	8	20	6	8
Reaction	16	4	20	4	32

Supplementary Table 4. Cost breakdown

Reagents		Prototype (current version)	Point of Care (hypothetical)
Inactivation (per x saliva samples)		$ 0.23	$ 0.23
Assay (per cartridge)			
	Primer mix	$0.42	$0.42
	Master mix	$5.51	$5.51
	Cas13	$0.17	$0.17
	T7 mix	$0.65	$0.65
	F/Q probe	$0.06	$0.06
	crRNA	$0.005	$0.005
	Total (1 cartridge)	**$6.82**	**$6.82**
Instrument			
	Electronic board with temperature controller	$2000	$50
	Syringe pump	$2100	$100
	Solenoid valves	$600	$300

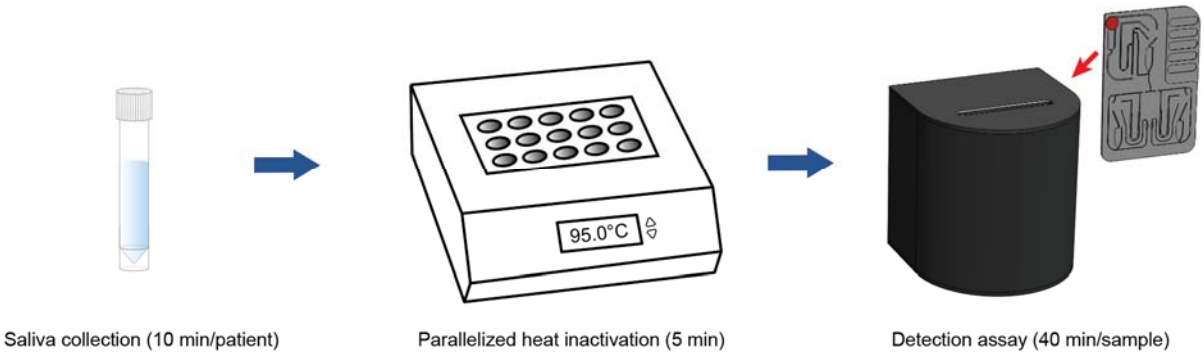
	Manifold components	$3500	$200
	Total (1 instrument)	**$8,200**	**$650**
			Production Volume - 500k
Cartridge	PMMA	$20	$2
	Adhesive	$5	$0.7
	Hydrophobic membranes	$2	$0.5
	Silicone rubber sheet	$6	$2
	Labor assembly	$75	$3
	Total (1 cartridge)	**$103**	**$8**
Detection			
	Eyepiece	$170	$100
	Camera	$550	$10
	Fluorescence filters	$25	$40

	Electronic Controllers/boards	$300	$30
	LED	$296	$15
	Other hardware (mounts, screws, etc)	$80	$40
	Total (1 detection system)	**$2583**	**~$235***

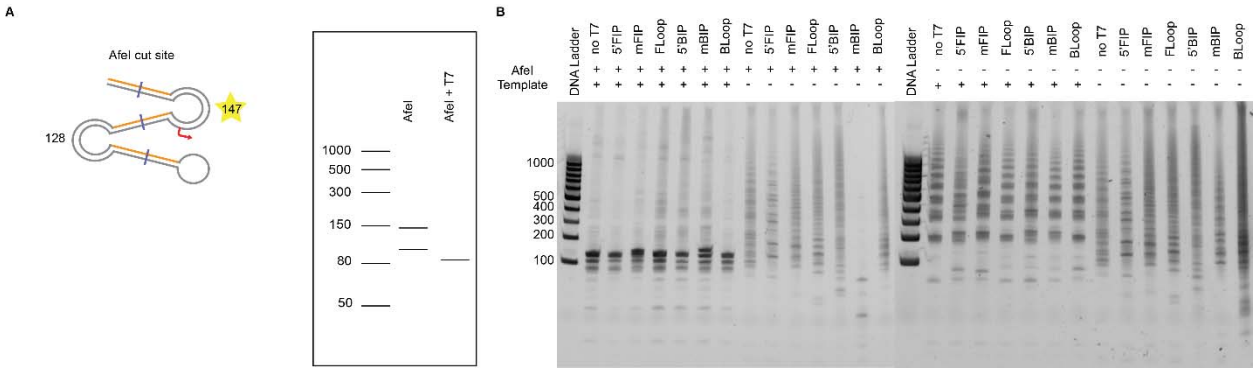
* An optical system designed to deliver similar detection limits but optimized for mass manufacture would have an estimated parts cost (very roughly, cost of goods sold) of less than $250. In a unit redesigned for production at volume, the camera sensor, high-power LED, and microcontrollers drop to under ~$10 each even in modest quantities. This leaves filters, optics, mounting and labor as the main cost drivers. Filter size can be reduced to the ~ 4mm diameter of the system aperture, reducing filter cost (on an areal basis) by > 25X to under ~ $40, and optics (~ $200) will also drop substantially at volume, approximating the low-cost objective they replace.

Supplementary Table 5. Clinical Saliva Sample Ct Values

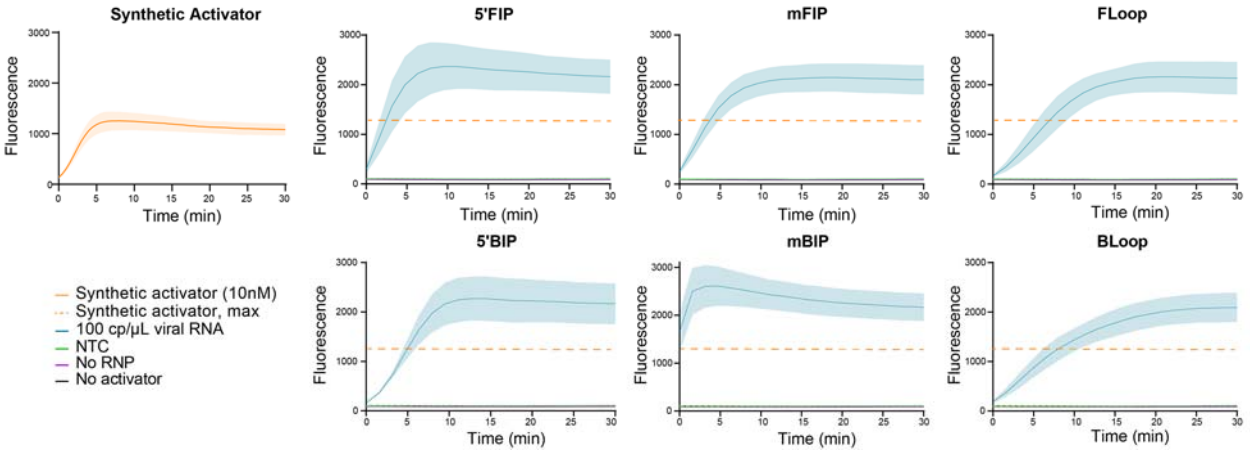
Ct		Donor ID
N	ACTB	
23.20	32.35	4
29.50	32.59	7
31.40	33.15	8
30.64	31.47	17
28.54	32.32	19
29.50	32.39	20
21.86	31.29	32
26.75	33.09	50
23.41	33.02	60
26.42	34.22	64
39.50	32.35	1
41.71	28.56	38
38.69	33.50	49



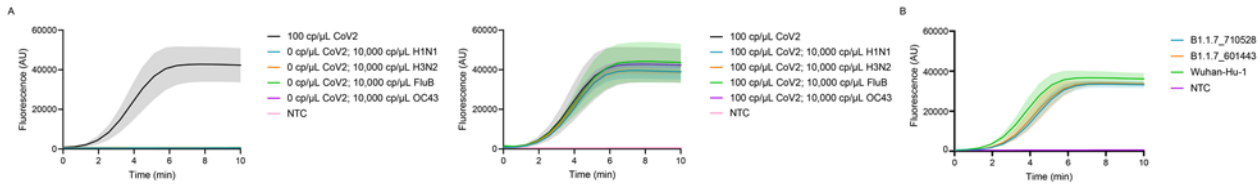
Supplemental Figure 1. Sample collection and inactivation workflow. Schematic of inactivation protocol. Clinical saliva could be collectively inactivated at 95C for 5 minutes before being processed individually onto the DISCoVER system.



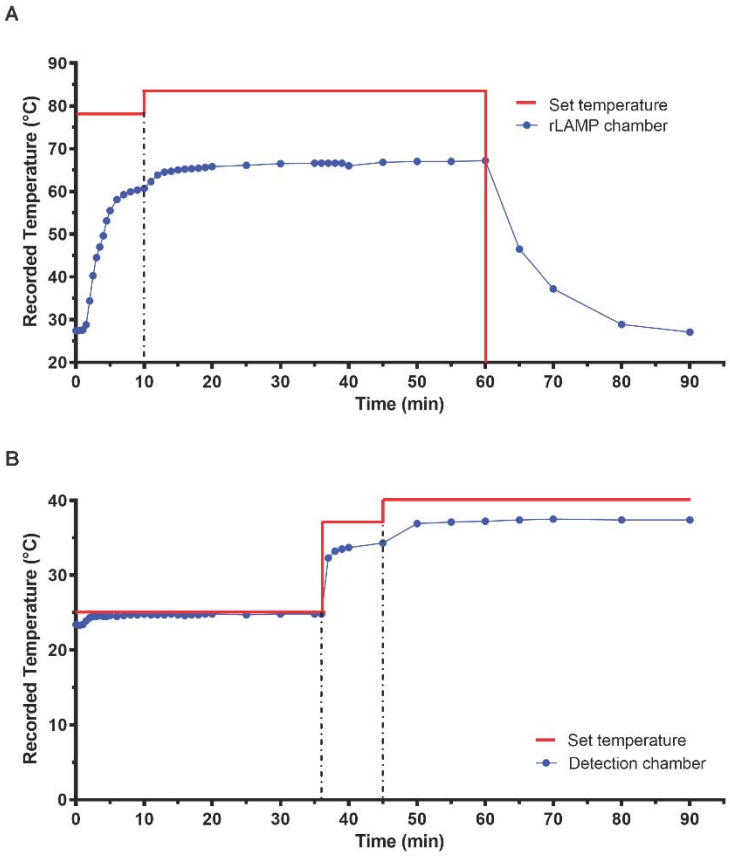
Supplemental Figure 2. Validation of rLAMP products. A. Schematic of primary mBIP rLAMP products and sizes upon AfeI digestion (left). Virtual gel depicting expected AfeI restriction digest bands (147 nt, 128 nt) and resulting transcription bands of mBIP rLAMP products. Only the 147 nt product is expected to contain the T7 promoter, and thus produce an ~85nt RNA product upon transcription. **B.** Restriction-digestion of each rLAMP product with each primer set containing the different T7 insertion positions.



Supplemental Figure 3. **Cas13 detection on all T7 insertion locations**, with T7 transcription and Cas13 detection occurring in the same reaction. The maximum fluorescence of the synthetic activator at 10 nM is plotted (dashed line). Values are mean ± SD with n = 3. NTC, no template control. RNP, ribonucleoprotein.



Supplemental Figure 4. Specificity testing of DISCoVER Assay. A. Detection of SARS-CoV-2 synthetic RNA in inactivated saliva in the presence of H1N2, H3N2, Influenza B, and human coronavirus OC43 synthetic viral RNA with N gene crRNA. Values are mean ± SD with n = 3. **B**. Detection of synthetic SARS-CoV-2 B.1.1.7. variants in saliva at 50 cp/µL with N gene crRNA. Values are mean ± SD with n = 3. NTC, no template control.



Supplemental Figure 5. Temperature characterization of the cartridge using a thermal calibration cartridge. Graph showing the temperature measured via integrated thermal-epoxy from the rLAMP chamber (**A**) and the detection chamber (**B**). On the x-axis, we showed the different timepoints at which the temperature was changed on the user interface.

Supplementary Video. Demonstration of the DISCoVER workflow. Test samples are collected into saliva collection tubes containing inactivation reagent. The sample is then heat-inactivated at 95ºC for 5 minutes and applied to the microfluidic cartridge via transfer pipette. The DISCoVER cartridge comes pre-loaded with reagents necessary for executing the test. Patient information is then inputted into the computer, the cartridge is inserted into the device, and the software is started to begin the test. During the run, data is displayed in real time and automatically resulted.