Supplementary Information – A new role for Biofoundries in rapid development and validation of automated SARS-CoV-2 clinical diagnostics

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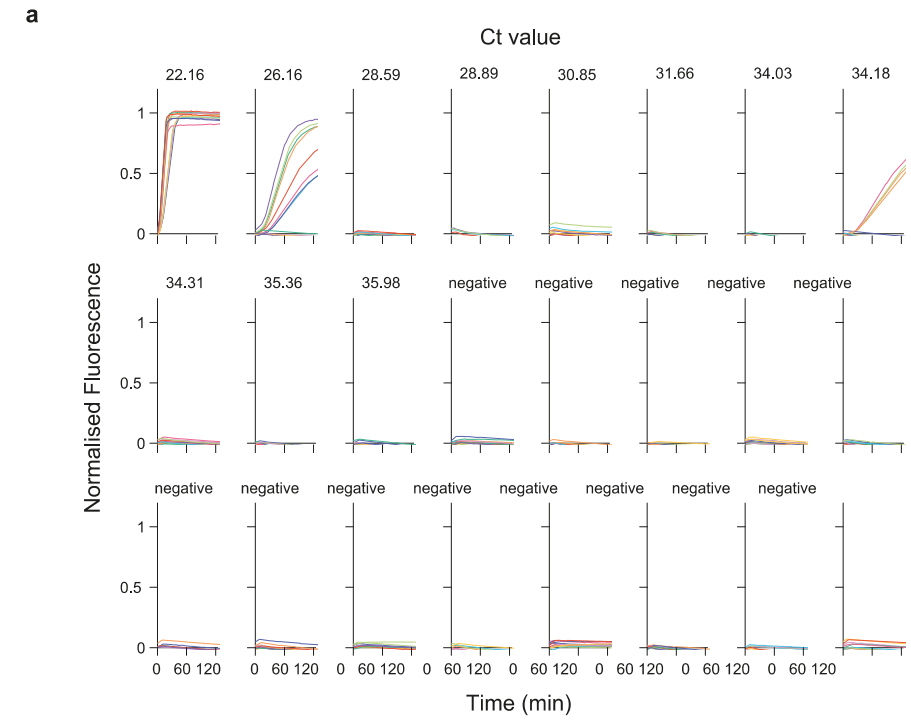
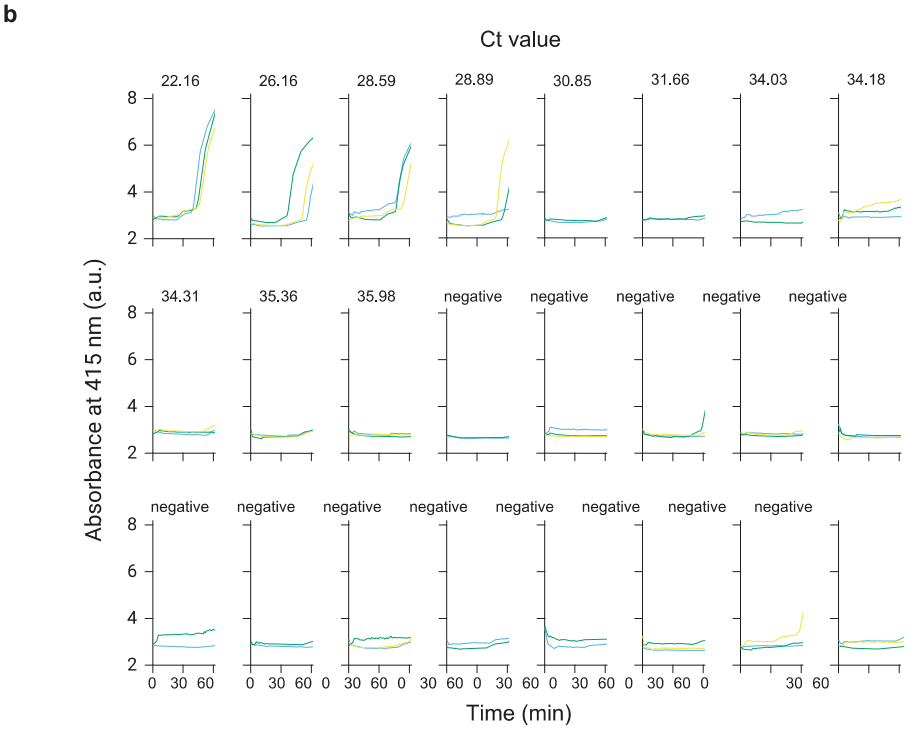
Supplementary Figures





Supplementary Figure 1: Patient Sample Validation for LAMP and RPA-CRISPR Assays. (a) Individual traces for the RPA-CRISPR patient assays for 24 patient samples. **(b)** Individual traces for the colorimetric LAMP patient assays for 24 patient samples.

Supplementary Tables

Supplementary Table 1: Patient Sample Validation for LAMP and RPA-CRISPR Assays.

		Copies per reaction			
Sample	**RT-qPCR (ct value)**	**PCR**	**LAMP**	**RPA-CRISPR**	**Viral copies/mL viral transport media**
1	22.16	6409.91	2563.96	3204.95	320495.36
2	26.16	422.20	168.88	211.10	21109.93
3	28.59	80.88	32.35	40.44	4044.14
4	28.89	65.96	26.38	32.98	3297.81
5	30.85	17.39	6.96	8.70	869.70
6	31.66	10.03	4.01	5.01	501.36
7	34.03	2.00	0.80	1.00	100.05
8	34.18	1.81	0.72	0.90	90.35
9	34.31	1.65	0.66	0.83	82.70
10	35.36	0.81	0.32	0.40	40.50
11	35.98	0.53	0.21	0.27	26.56
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Copies per reaction are calculated using the line of best fit from the standard curve in Figure 2b (y = -3.386x + 35.06) and differ based on input volume between RT-qPCR (5 µL), LAMP (2 µL) and RPA-CRISPR (2.5 µL). The number of viral copies per millilitre of transport media is assuming 100% extraction efficiency with a sample volume of 200 µL and elution volume of 50 µL. Blocks highlighted in yellow were detected in the LAMP or RPA-CRISPR assays.

Supplementary Table 2: Summary of available diagnostic methodologies for high-throughput testing.

Assay	Commercial Reagents	Limit of Detection (gene)	Time to Result	HT Techniques	Advantages	Disadvantages
RT-qPCR (this work)	NEB (E3005L), ThermoFisher (A15299 or 4444432)	1 copy per reaction (N)	150 minutes	Automated RNA extraction, Echo setup	Highest sensitivity	Thermocycling
RT-LAMP (this work)	NEB (M1800L)	~30 copies per reaction (N)	100 minutes	Automated RNA extraction, Echo setup	Isothermal amplification	Potential non-specific amplification No multiplexing in a single reaction
RT-PCR-CRISPR (this work)	RT-PCR: NEB (E3005L) LwCas13a: In-house	~2.5 copies per reaction (N)	170 minutes	Automated RNA extraction, Echo setup	Plate reader instead of qPCR results in higher throughput	Thermocycling More complicated setup
RPA-CRISPR (this work)	RPA: TwistDx (TALQBAS01) LwCas13a: In-house	~200 copies per reaction (ORF1ab)	150-210 minutes	Automated RNA extraction, Echo setup	Higher throughput than RTqPCR	Not as sensitive as alternatives More complicated assay setup
LAMP-CRISPR (IDT/Sherlock Biosciences)	IDT/Sherlock Biosciences (10006968)	360 copies per reaction (ORF1ab) 72 copies per reaction (N)	120 minutes	Multichannel setup	Short time to result	Very complicated assay setup Low throughput (190 per shift per operator)

Supplementary Table 3: Primer and Probe Sequences for qPCR/RT-ddPCR

Figure	Name	Forward Primer	Reverse Primer	Probe
1d, 2a, 2b, 2c, 4b, 5b, 5c	2019-nCoV_N1	GACCCCAAAATCAG CGAAAT	TCTGGTTACTGCCAGTTGA ATCTG	FAM - ACCCCGCATTACGTTTGGTGGACC - BHQ1
2c	2019-nCoV_N2	TTACAAACATTGGCC GCAAA	GCGCGACATTCCGAAGAA	FAM - ACAATTTGCCCCCAGCGCTTCAG - BHQ1
2c	2019-nCoV_N3	GGGAGCCTTGAATA C ACCAAAA	TGTAGCACGATTGCAGCA TTG	FAM - AYCACATTGGCACCCGCAATCCTG - BHQ1

Supplementary Table 4: Primers and oligonucleotides for CRISPR-Cas13a reactions

Figure	Name	Forward primer/oligonucleotide	Reverse primer/oligonucleotide
3b, 4c	2019-nCoV_N1 IVT crRNA	GTATAATACGACTCACTATAGG	ACCCCGCATTACGTTTGGTGGACCGTTTTAG TCCCCTTCGTTTTTGGGGTAGTCTAAATCCCT ATAGTGAGTCGTATTATAC
3b	2019-nCoV_N2 IVT crRNA	GTATAATACGACTCACTATAGG	CACAATTTGCCCCCAGCGCTTCAGCGGTTTT AGTCCCCTTCGTTTTTGGGGTAGTCTAAATC CCTATAGTGAGTCGTATTATAC
3b	2019-nCoV_N3 IVT crRNA	GTATAATACGACTCACTATAGG	GATCACATTGGCACCCGCAATCCTGCTGTTT TAGTCCCCTTCGTTTTTGGGGTAGTCTAAAT CCCTATAGTGAGTCGTATTATAC
5d	LwCas13a orf1ab IVT crRNA	GTATAATACGACTCACTATAGG	ATAGTTTAAAAATTACAGAAGAGGTTGGGT TTTAGTCCCCTTCGTTTTTGGGGTAGTCTAAA TCCCTATAGTGAGTCGTATTATAC
3b	2019-nCoV_N1 PCR amplification T7	GAAATTAATACGACTCACTATAGGGACCCCAAA ATCAGCGAAAT	TCTGGTTACTGCCAGTTGAATCTG
3b	2019-nCoV_N2 PCR amplification T7	GAAATTAATACGACTCACTATAGGGTTACAAACA TTGGCCGCAAA	GCGCGACATTCCGAAGAA
3b	2019-nCoV_N3 PCR amplification T7	GAAATTAATACGACTCACTATAGGGAGCCTTGA ATACACCAAAA	TGTAGCACGATTGCAGCATTG
5d	Orf1ab RPA amplification T7[1]	GAAATTAATACGACTCACTATAGGGCGAAGTTG TAGGAGACATTATACTTAAACC	TAGTAAGACTAGAATTGTC TACATAAGCAGC

Supplementary Table 5: Primer and Probe Sequences for LAMP Reactions

Figure	Name	Sequence	Final Concentration
3d, 5e	GeneN-A-F3	TGGCTACTACCGAAGAGCT	1.6 µM
	GeneN-A-B3	TGCAGCATTGTTAGCAGGAT	1.6 µM
	GeneN-A-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	0.2 µM
	GeneN-A-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	0.2 µM
	GeneN-A-LF	GGACTGAGATCTTTCATTTTACCGT	0.4 µM
	GeneN-A-LB	ACTGAGGGAGCCTTGAATACA	0.4 µM

Primer sequences from Zhang *et al*.[2]

References

1. Metsky, H. C., Freije, C. A., Kosoko-Thoroddsen, T.-S. F., Sabeti, P. C. & Myhrvold, C. CRISPR-based COVID-19 surveillance using a genomically-comprehensive machine learning approach. *bioRxiv* 2020.02.26.967026 (2020) doi:10.1101/2020.02.26.967026.

2. Zhang, Y. *et al.* Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP. *medRxiv* **2**, 2020.02.26.20028373 (2020).