Supplementary information to

Digital Microfluidic RT-qPCR Cartridge for Detection of SARS-CoV-2 and Discrimination of Delta and Omicron Variants

Kuan-Lun Ho [1], Jing Ding [1], Jia-Shao Fan [2], Wai Ning Tiffany Tsui [3], Jianfa Bai [3], and Shih-Kang Fan [1,*]

[1] Department of Mechanical and Nuclear Engineering, Kansas State University, Manhattan, KS 66506, USA
[2] Department of Electrical and computer Engineering, Kansas State University, Manhattan, KS 66506, USA
[3] Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS 66506, USA
* Correspondence: skfan@ksu.edu

Supplementary Information includes:

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Figure S2. The uncropped initial (cycle 0) and end-point (cycle 45) fluorescence images of RT-qPCR for clinical analog samples representing (a) wildtype infection, (b) Delta variant infection, (c) Omicron variant infection, and (d) no infection.

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Table S1. Primers and probes used in this study.

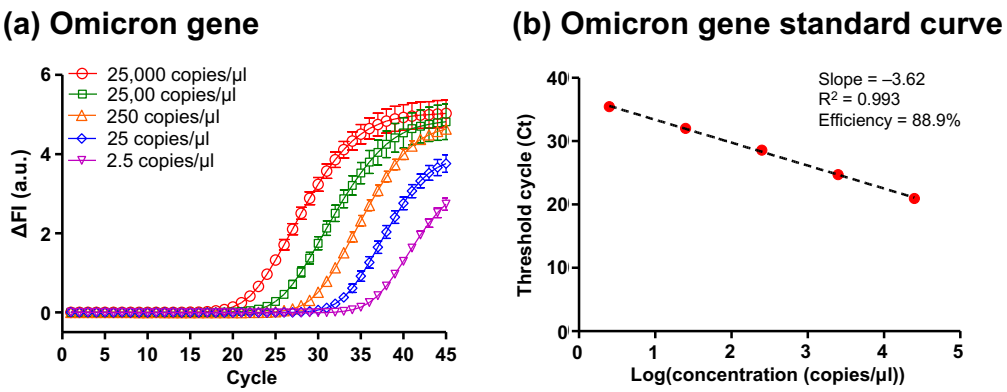
Primer/probe name	Sequence (5′-3′)	Amplicon size
2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT	72 bp
2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG	
2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC	
SARS2-dF	CCACAAAAACAACAAAAGTTGG	78 bp for Delta 84 bp for non-Delta
SARS2-dR	TGAGAGACATATTCAAAAGTGCAA	
SARS2-dPr	FAM-ATAAACTCCACTTTCCA-MGB	
SARS2-wPr	AGAATAAACTCTGAACTCACTTTCCATCC	
OmN-F	GGACCCTCAGATTCAACTGG	86 bp for Omicron 95 bp for non-Omicron
OmN-R	GCAGTATTATTGGGTAAACCTTGG	
OmNm-Pr	FAM-ATCGCGCCCCACCATTCT	
OmNw-Pr	CGCCCCACTGCGTTCTCC	
18S-F	GGAGTATGGTTGCAAAGCTGA	100 bp
18S-R	GGTGAGGTTTCCCGTGTTG	
18S-Pr	FAM-AAGGAATTGACGGAAGGGCA	

Table S2. RT-qPCR reaction mix solutions for detection and discrimination of SARS-CoV-2 wildtype, Delta variant, and Omicron variant.

Droplet (1.5 μl)		A	B	C	D	E
Target Gene		N1	Delta	Omicron	18S	NTC
RT-qPCR Component		Volume (μl)				
TaqPath™ 1-Step RT-qPCR Master Mix (4X)		5	5	5	5	5
Nuclease free water w/ 0.4% (v/v) tween-20		5	5	5	5	5
Nuclease free water		3	2.5	2.25	3	0.75
N1	N1 Primer mix (10 μM)	1	0	0	0	1
	N1-P (10 μM, FAM)	1	0	0	0	1
Delta	SARS2-d Primer mix (10 μM)	0	1	0	0	1
	SARS2-dPr (10 μM, FAM)	0	0.5	0	0	0.5
	SARS2-wPr (15 μM)	0	1	0	0	1
Omicron	OmN Primer mix (10 μM)	0	0	1	0	1
	OmNm-Pr (10 μM, FAM)	0	0	0.75	0	0.75
	OmNw-Pr (15 μM)	0	0	1	0	1
18S	18S Primer mix (10 μM)	0	0	0	1	1
	18S-Pr (10 μM, FAM)	0	0	0	1	1
Sample	SARS-CoV-2 RNA (WT, Delta, or Omicron, 80 copies/μl)	2.5	2.5	2.5	2.5	0
	18S rDNA (7.44 pg/μl)	2.5	2.5	2.5	2.5	0
Total volume		**20**	**20**	**20**	**20**	**20**

Table S3. RT-qPCR reaction mix solutions for detection of SARS-CoV-2 Omicron variant.

RT-qPCR Component	Volume (µl)
TaqPath™ 1-Step RT-qPCR Master Mix (4X)	5
Nuclease free water w/ 0.4% (v/v) tween-20	5
Nuclease free water	3.25
OmN Primer mix (10 µM)	1
OmNm-Pr (10 µM, FAM)	0.75
Omicron RNA Template (100,000 copies/µl to 10 copies/µl)	5
Total	**20**



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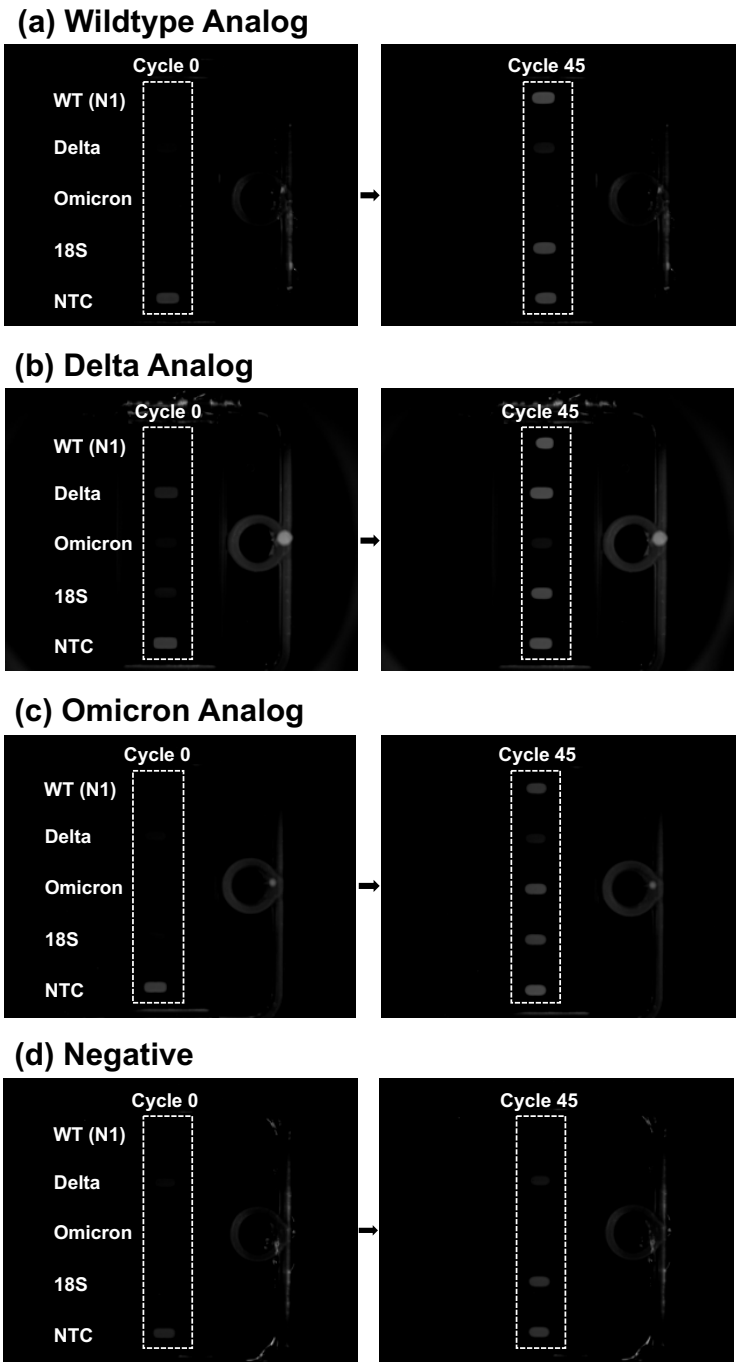

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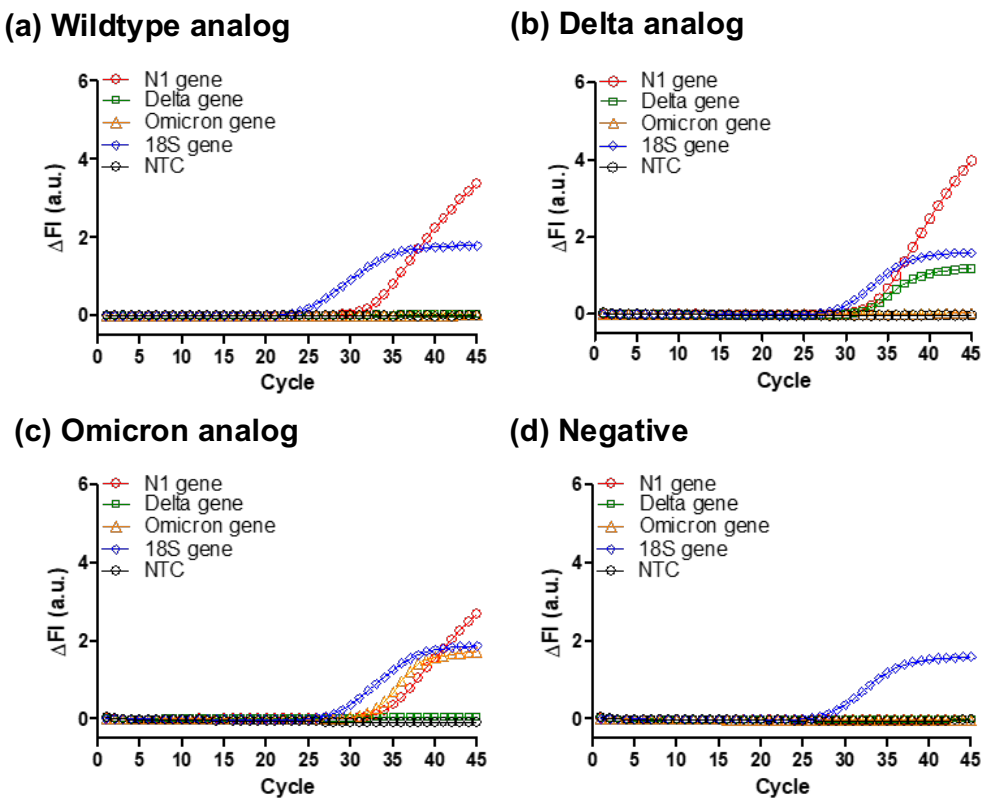

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