

SUPPLEMENTAL TABLES

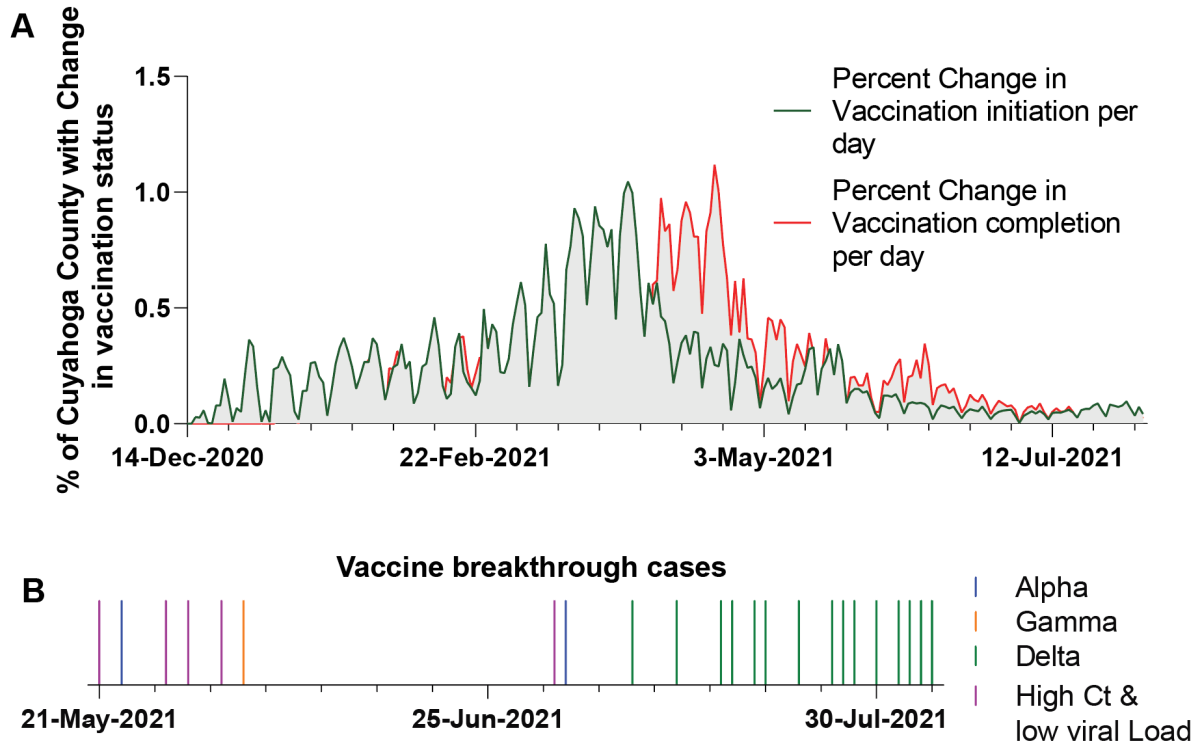
Plate	Primer Name	PCR Conditions	Primer Sequence	Citation/Source
First plate	ORF1ab	Per Manufacturer's instruction		Taqpath COVID-19 Combo assay
	N gene	Per Manufacturer's instruction		Taqpath COVID-19 Combo assay
	S gene	Per Manufacturer's instruction		Taqpath COVID-19 Combo assay
Second Plate	ORF1del: Δ3675-3677	360 nM of each primer 80 nM of each probe 25°C 2 min x1 52°C 15 min x1 94°C 2 min x1 (94°C 15 sec, 57°C 40 sec, 68°C 20 sec) x45	Forward: TGCCTGCTAGTTGGGTGATG Reverse: TGCTGTCATAAGGATTAGTAACACT Probe: 5CY5--GTTTGTCTG /TAO/ GTTTTAAGCTAAAAGACTGTG--3IAbrQSp	[8]
	S protein: N501Y	360 nM of each primer 80 nM of each probe 25°C 2 min x1 52°C 15 min x1 94°C 2 min x1 (94°C 15 sec, 57°C 40 sec, 68°C 20 sec) x45	Forward: CTGAAATCTATCAGGCCGGTA Reverse: GAAAGTACTACTACTCTGTATGG Probe: 56-FAM--TTTCCAACCCACTTATGGT--3BHQ_1	[9]
	S protein: E484K	400 nM of each primer 50°C 10 min x1 95°C 1 min x1 (95°C 2 min, 55°C 15 sec) x40	Forward: ACACCTTGTAATGGTGTTA Reverse: CTGGTGCATGTAGAAGTTCA Probe: SYBR Green	This manuscript
	S protein: L452R	360 nM of each primer 80 nM of each probe 25°C 2 min x1 52°C 15 min x1 94°C 2 min x1 (94°C 15 sec, 57°C 40 sec, 68°C 20 sec) x45	Forward: CTCTCTCAAAGGTTTGAGATTAGACT Reverse: CTTGATTCTAAGTTGGTGTTAA Probe: HEX--CCTAAACAATCTATACCGGTAATT--3BHQ_1	[9]

Strain	Strain Type	# of GISAID sequences	ORF1del(%)	S:N501Y (%)	S: E484K (%)	S: L452R (%)
Alpha (B.1.1.7)	VoC	115403	96.5	97.8	0.8	0.1
Beta (B.1.351)	VoC	2988	90.9	87.6	87.2	0.3
Gamma (P.1)	VoC	18685	97.6	95.3	94.9	0.0
Delta (B.1.617.2, AY.3)	VoC	418169	0.1	0.1	0.0	98.0
Eta (B.1.525)	Vol	560	95.4	0.4	97.3	0.0
Ito (B.1.526)	Vol	1949	96.9	0.1	78.0	0.0

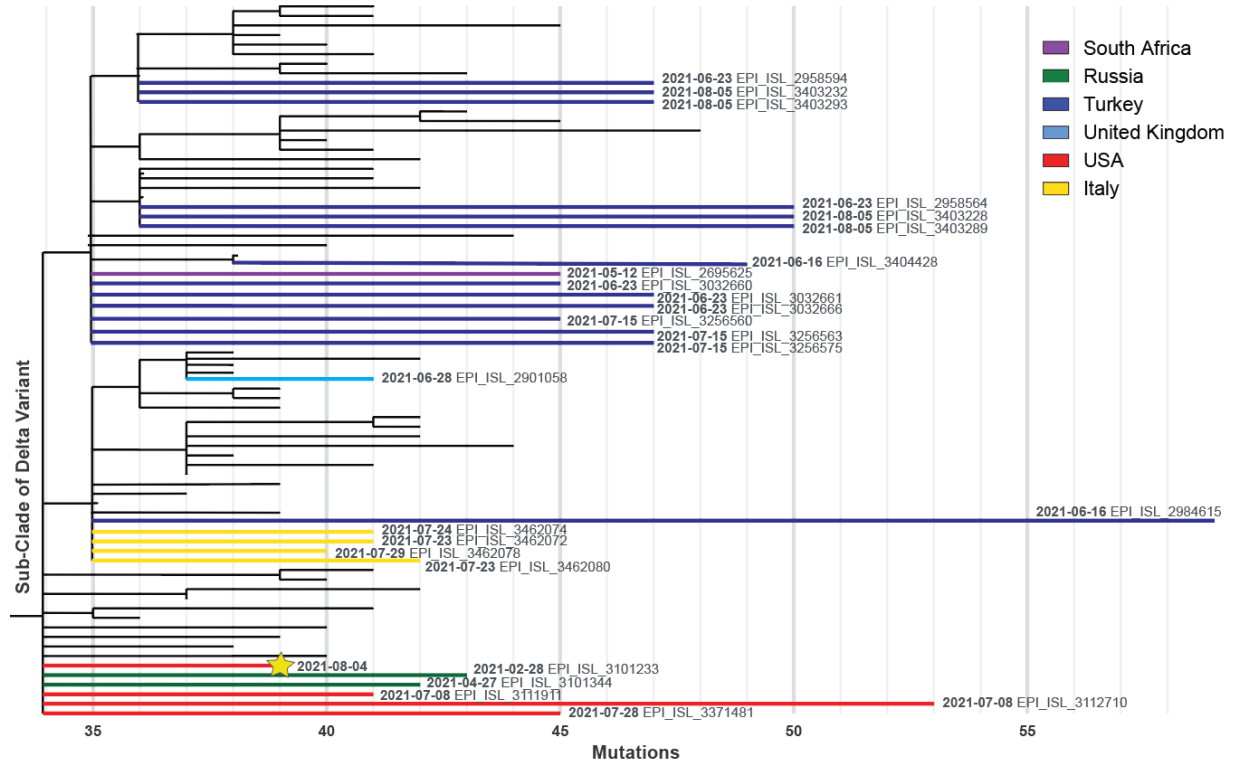
Supplemental Table 3: Example ruleset for calling of Variants on qPCR-based Screen

ORF1ab	N gene	S gene	ORF1 del: Δ3675-3677	S: N501Y	S: E484K	S: L452	<u>Interpretation</u>
CT< 40	CT<40	(S gene CT) – (N gene CT) =< 6	CT<40	CT<40	(E484K CT) – (N gene CT) =<5	CT<40	
TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	Alpha (B.1.1.7)
TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	Alpha (B.1.1.7+E484K)
TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	Gamma or Beta
TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	Delta
TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	Eta
TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	Iota
TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	B.1.526.1
TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	B.1.526.2
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	B.1.621
TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	P.2

SUPPLEMENTAL FIGURES



Supplemental Figure 1: A) Cuyahoga County vaccination initiation and completion rates as a percentage of the population. B) Incidence of positive SARS-CoV-2 tests over time per variant. Each line indicates one positive result. Colors indicate the variant identified or high Ct, respectively.



Supplemental Figure 2: Subset of Nextclade phylogenetic tree containing the Delta+E484K sample (indicated with star) and most closely related Delta+E484K samples. Delta variant reference samples are shown in black lines. For Delta+E484K samples, country of origin labeled with colors per legend, date sample was collected in bold, and Accession ID's indicated.

REFERENCES (as per main text)

- Vogels, C.B.F., et al., Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *PLoS Biol*, 2021. 19(5): p. e3001236.
- Wang, H., et al., Multiplex SARS-CoV-2 Genotyping Reverse Transcriptase PCR for Population-Level Variant Screening and Epidemiologic Surveillance. *J Clin Microbiol*, 2021. 59(8): p. e0085921.