

## Supporting Information

### Multiplex Assays Enable Simultaneous Detection and Identification of SARS-CoV-2 Variants of Concern in Clinical and Wastewater Samples

Yanming Liu,<sup>1</sup> Teresa Kumblathan,<sup>1</sup> Michael A. Joyce,<sup>2</sup> D. Lorne Tyrrell,<sup>2</sup> Graham  
Tipples,<sup>2,3</sup> Xiaoli Pang,<sup>3,4</sup> Xing-Fang Li,<sup>1\*</sup> X. Chris Le<sup>1\*</sup>

- 1 Division of Analytical and Environmental Toxicology, Department of Laboratory  
Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta,  
Edmonton, Alberta, Canada T6G 2G3
- 2 Li Ka Shing Institute of Virology, Department of Medical Microbiology and  
Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton,  
Alberta, Canada T6G 2E1
- 3 Provincial Laboratory for Public Health, Alberta Precision Laboratories, University of  
Alberta Hospitals, 8440-112 St, Edmonton, Alberta, Canada T6G 2J2
- 4 Department of Laboratory Medicine and Pathology, Faculty of Medicine and  
Dentistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

\* Corresponding authors. [xc.le@ualberta.ca](mailto:xc.le@ualberta.ca) and [xingfang.li@ualberta.ca](mailto:xingfang.li@ualberta.ca)

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**Table S1.** Amino acid changes in the spike protein of variants of concern (VOCs) designated by the World Health Organization (WHO)

WHO label (Lineage)	Position of mutated amino acids in Spike protein*	
<b>Alpha</b> (B.1.1.7 and descendent lineages)	<b>HV69/70 del</b> , Y114 del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H <b><u>E484K, L452R</u></b>	
<b>Beta</b> (B.1.351 and descendent lineages)	D80A, D215G, L242-L244del, <b>K417N</b> , E484K, N501Y, D614G, A701V, <b><u>L18F</u></b>	
<b>Gamma</b> (P.1 and descendent lineages)	L18F, T20N, P26S, D138Y, R190S, <b>K417T</b> , E484K, N501Y, D614G, H655Y, T1027I, V1176F, <b><u>P681H</u></b>	
<b>Delta</b> (B.1.617.2 and descendent lineages)	T19R, E156-F157del, R158G, L452R, <b>T478K</b> , D614G, <b>P681R</b> , D950N, <b><u>T417N (in delta plus), E484K</u></b>	
<b>Omicron</b> (B.1.1.529)	<b>Common mutations of BA.1 to BA.5</b>	G142D, G339D, S375F, <b>K417N</b> , N440K, S373P, S477N, <b>T478K</b> , E484A, Q493K, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
	<b>Unique mutations of BA.1</b>	A67V, <b>HV69/70 del</b> , T95I, Δ143-145 del, N211del, L212I, ins214EPE, S371L, G446S, G496S, T547K, N856K, L981F
	<b>Unique mutation of BA.2</b>	T19I, LPPA24S, V213G, S371F, T376A, D405N, R408S
	<b>BA.3</b>	<b>A67V, HV69/70 del, T95I, Δ143-145, Δ211, L212I, G446S, S371F, D405N</b> (share 7 unique mutations with BA.1 and 2 with BA.2)
	<b>BA.4</b>	<b>BA.2 + HV69/70 del, L452R, F486V, Q493 reversion</b>
	<b>BA.5</b>	<b>BA.2 + HV69/70 del, L452R, F486V, Q493 reversion</b>

\* Mutations shown in **bold** and underlined are only found in descendent lineages. Mutations chosen as targets in multiplex RT-qPCR assays are shown in **bold** and **red color**. Mutations in **green color** indicate unique mutations shared by Omicron sub-variants BA.1 and BA.3. Mutations in **purple color** are unique mutations shared by Omicron sub-variants BA.2 and BA.3. Mutations in **blue color** are additional mutations shared by Omicron sub-variants BA.4 and BA.5 relative to BA.2. All the information in the table was adapted from WHO (<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>) and GISAID (<https://www.gisaid.org/>)

**Table S2.** Optimal RT-qPCR conditions for singleplex and multiplex assays for the detection of five targets: HV69/70 deletion, and K417N, K417T, T478K, and P681R mutations

Target	Singleplex RT-qPCR				Assay	Multiplex RT-qPCR			
	F-primer (nM)	R-primer (nM)	Probe (nM)	Annealing T (°C) *		F-primer (nM)	R-primer (nM)	Probe (nM)	Annealing T (°C) *
<b>HV69/70 deletion</b>	100	800	250	61		100	800	250	
<b>K417N</b>	100	800	500	60	ABG	100	800	500	61
<b>K417T</b>	100	800	500	60		100	800	500	
<b>T478K</b>	400	800	250	64	Delta	400	800	250	64
<b>P681R</b>	200	800	250	60		100	800	250	

\*T represents temperature.

**Table S3.** Performance comparison between singleplex and multiplex RT-qPCR assays for the five targets

<b>Target</b>	<b>Template (Copies)</b>	<b>10<sup>2</sup> (Ct value)</b>	<b>10<sup>4</sup> (Ct value)</b>	<b>10<sup>6</sup> (Ct value)</b>
<b>HV 69/70 deletion</b>	Singleplex Ct	34.65	27.92	20.62
	Multiplex (ABG) Ct	34.61	27.48	19.82
	<b>ΔCt value (single Ct - multi Ct)</b>	<b>0.04</b>	<b>0.44</b>	<b>0.8</b>
<b>K417N</b>	Singleplex Ct	34.66	26.77	20.10
	Multiplex (ABG) Ct	34.50	26.40	19.21
	<b>ΔCt value</b>	<b>0.16</b>	<b>0.33</b>	<b>0.89</b>
<b>K417T</b>	Singleplex Ct	33.73	26.90	20.47
	Multiplex (ABG) Ct	34.15	27.21	19.97
	<b>ΔCt value</b>	<b>-0.42</b>	<b>-0.31</b>	<b>0.5</b>
<b>T478K</b>	Singleplex Ct	33.53	26.32	18.99
	Multiplex (delta) Ct	32.72	25.54	18.75
	<b>ΔCt value</b>	<b>0.81</b>	<b>0.78</b>	<b>0.24</b>
<b>P681R</b>	Singleplex Ct	33.78	26.08	19.35
	Multiplex (delta) Ct	32.89	25.99	19.20
	<b>ΔCt value</b>	<b>0.89</b>	<b>0.09</b>	<b>0.15</b>

ΔCt between singleplex and multiplex assays was <1 for all five targets and three template concentrations, suggesting that both the singleplex and multiplex assays perform similarly.

**Table S4.** Analytical sensitivity of the ABG multiplex RT-qPCR assay for the detection of the Alpha, Beta, and Gamma variants of SARS-CoV-2

RNA	Copies for each variant RNA per reaction	Average Ct (SD) <sup>a</sup>			Detected/tested (%)		
		HV69/70 deletion (Alpha)	K417N (Beta)	K417T (Gamma)	HV69/70 deletion (Alpha)	K417N (Beta)	K417T (Gamma)
Alpha Beta Gamma	250	30.15 (0.36)	32.07 (0.62)	32.47 (0.09)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	125	31.12 (0.18)	32.65 (0.68)	33.81 (0.54)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	62	32.23 (0.08)	33.98 (0.42)	35.30 (0.14)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	31	33.45 (0.39)	34.39 (0.50)	35.38 (0.93)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	16	33.57 (0.13)	35.97 (0.84)	37.82 (0.48)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	8	34.15 (0.67)	37.14 (0.48)	38.55 (0.90)	10/10 (100%)	<b>10/10 (100%)</b>	<b>10/10 (100%)</b>
	4	36.67 (1.1)	37.80 (1.07)	38.91 (0.98)	<b>10/10 (100%)</b>	9/10 (90%)	9/10 (90%)

<sup>a</sup> SD denotes one standard deviation.

The results in **red color** indicate the limit of detection (LOD) for the three targets. The LOD was defined as the lowest RNA copy number detected in all 10 replicates. The LOD for detecting Alpha (HV 69/70 deletion) was 4 copies of RNA per reaction. The LOD for detecting Beta (K417N) and Gamma (K417T) was 8 copies of RNA per reaction.

**Table S5.** Analytical sensitivity of the Delta multiplex RT-qPCR assay for the detection of the Delta variant

RNA	Copies/ reaction	Average Ct (SD) <sup>a</sup>		Detected/tested (%)	
		T478K	P681R	T478K	P681R
Delta	<b>250</b>	33.87 (0.24)	34.21 (0.16)	6/6 (100%)	6/6 (100%)
	<b>125</b>	34.70 (0.44)	35.13 (0.47)	6/6 (100%)	6/6 (100%)
	<b>62.5</b>	35.58 (0.69)	36.00 (0.38)	6/6 (100%)	6/6 (100%)
	<b>31</b>	36.10 (0.45)	36.71 (0.52)	6/6 (100%)	6/6 (100%)
	<b>16</b>	36.73 (0.60)	37.05 (0.34)	<b>10/10 (100%)</b>	<b>10/10 (100%)</b>
	<b>8</b>	38.55 (1.00)	38.64 (0.98)	4/10 (40%)	4/10 (40%)

<sup>a</sup> SD denotes one standard deviation.

The results in **red color** indicate the limit of detection (LOD). The LOD was defined as the lowest RNA concentration detected in all 10 replicates. The LOD for detecting the Delta target with either the T458K or P681R mutation was 16 copies of RNA per reaction.

**Table S6.** Results of repeated analyses of each target at different concentrations using two multiplex RT-qPCR assays

Assay	Target	Copies/ reaction	Intra-assay variability			Inter-assay variability		
			Ct value (mean)	SD <sup>a</sup>	%CV <sup>b</sup>	Ct value (mean)	SD	%CV
<b>ABG multiplex</b>	<b>HV69/70 deletion</b>	5×10 <sup>4</sup>	21.43	0.14	0.6	21.48	0.01	1.15
		8	34.28	0.39	1.1	34.77	0.67	1.9
	<b>K417N</b>	5×10 <sup>4</sup>	24.06	0.18	1.8	23.58	0.52	2.2
		8	36.78	0.10	0.3	36.88	0.55	1.5
	<b>K417T</b>	5×10 <sup>4</sup>	25.21	0.11	0.4	25.12	0.30	1.2
		8	37.30	0.15	0.4	38.45	0.90	2.3
<b>Delta multiplex</b>	<b>T478K</b>	5×10 <sup>4</sup>	26.81	0.16	0.5	26.85	0.31	1.1
		16	36.73	0.60	1.7	37.05	1.00	2.7
	<b>P681R</b>	5×10 <sup>4</sup>	27.25	0.16	0.6	27.42	0.27	0.9
		16	35.50	0.34	0.9	35.86	0.68	1.9

<sup>a</sup> SD denotes one standard deviation. <sup>b</sup> %CV indicates percent coefficient variation.



**Table S7.** List of Ct values from testing of Omicron sub-variant BA.1 in RNA extracted from nasopharyngeal swab samples. Ct values were obtained using the multiplex ABG assay targeting the HV69/70 deletion and K417N mutation. For comparison, Ct values provided by Alberta Precision Laboratories (APL) were from a multiplex RT-PCR assay targeting the E gene and the HV69/70 mutation and N501Y mutation in the S gene.

Sample number	HV69/70 deletion	K417N	E gene	HV69/70 deletion	N501Y
	This study			Provided by APL	
1	21.82	23.39	21.16	22.46	21.70
2	37.00	37.30	35.66	37.20	35.87
3	36.03	37.67	34.59	36.69	34.42
4	27.99	28.94	27.61	29.37	28.30
5	29.32	30.66	29.47	31.02	29.99
6	30.49	31.15	30.11	31.82	30.75
7	28.71	30.51	27.25	28.94	27.91
8	37.97	38.00	35.77	38.57	36.63
9	36.25	37.28	34.79	36.31	34.61
10	35.54	35.27	33.66	35.00	33.55
11	25.45	27.06	24.91	26.16	25.29
12	25.18	25.23	24.63	25.90	24.94
13	26.56	26.93	25.66	27.02	25.95
14	32.91	32.40	31.17	32.49	31.11
15	21.81	20.62	19.52	20.96	20.19
16	23.14	21.08	20.22	21.63	20.85
17	20.67	19.52	18.74	20.00	19.40
18	25.92	26.50	23.78	24.95	24.06
19	36.53	38.45	35.73	36.79	36.01
20	31.13	32.07	32.68	34.11	32.76
21	26.23	26.64	23.99	25.46	24.46
22	23.50	24.34	21.84	23.12	22.31
23	37.08	37.16	35.81	37.85	36.58
24	27.64	27.37	26.53	28.00	26.92
25	26.18	26.08	23.77	25.21	24.00
26	21.98	22.86	20.31	21.58	20.79
27	26.63	26.67	24.64	26.19	25.18
28	31.80	32.57	29.76	31.46	30.36
29	36.94	37.20	35.07	36.89	35.14
30	39.97	40.00	41.23	38.91	37.01
31	35.82	37.68	35.21	37.27	35.65
32	33.85	34.90	32.91	34.62	32.84
33	31.41	32.85	31.10	32.89	31.41
34	32.63	35.32	32.61	33.97	32.91
35	34.67	36.03	33.81	35.29	34.23
36	23.76	24.39	22.52	23.58	22.83
37	26.73	26.92	25.58	27.19	26.15
38	32.30	32.77	30.78	32.70	31.62
39	29.41	30.97	28.56	30.40	29.21
40	36.80	37.66	42.07	38.71	36.86
41	35.82	36.54	34.63	36.17	34.89
42	31.05	31.85	29.80	32.03	30.84

43	<b>30.23</b>	<b>31.71</b>	30.74	<b>32.04</b>	30.97
44	<b>23.43</b>	<b>22.75</b>	21.73	<b>23.13</b>	22.09
45	<b>22.82</b>	<b>22.82</b>	21.32	<b>22.66</b>	21.66
46	<b>28.34</b>	<b>27.67</b>	26.91	<b>28.38</b>	27.11
47	<b>21.85</b>	<b>21.58</b>	20.27	<b>21.52</b>	20.71
48	<b>23.48</b>	<b>23.63</b>	22.64	<b>24.01</b>	23.15
49	<b>28.95</b>	<b>29.18</b>	27.59	<b>29.18</b>	28.15
50	<b>34.51</b>	<b>34.25</b>	33.99	<b>35.29</b>	33.99
51	<b>22.06</b>	<b>22.00</b>	20.88	<b>22.16</b>	21.45
52	<b>23.01</b>	<b>22.76</b>	21.80	<b>23.11</b>	22.24
53	<b>24.55</b>	<b>23.95</b>	23.28	<b>24.61</b>	23.66
54	<b>23.96</b>	<b>23.87</b>	22.68	<b>24.15</b>	23.17
55	<b>20.82</b>	<b>20.28</b>	18.99	<b>20.08</b>	19.35
56	<b>27.01</b>	<b>26.80</b>	25.65	<b>27.12</b>	26.04
57	<b>28.64</b>	<b>29.34</b>	27.57	<b>29.47</b>	28.35
58	<b>34.18</b>	<b>35.77</b>	34.38	<b>36.24</b>	35.10
59	<b>27.29</b>	<b>28.81</b>	26.69	<b>28.38</b>	27.24
60	<b>19.82</b>	<b>20.23</b>	18.47	<b>19.78</b>	18.92
61	<b>30.08</b>	<b>34.87</b>	34.39	<b>35.78</b>	34.20
62	<b>30.12</b>	<b>29.24</b>	34.91	<b>36.22</b>	34.71
63	<b>23.03</b>	<b>22.86</b>	21.67	<b>23.08</b>	22.21
64	<b>25.43</b>	<b>25.32</b>	24.43	<b>25.70</b>	24.85
65	<b>21.91</b>	<b>20.94</b>	20.15	<b>21.52</b>	20.82
66	<b>35.40</b>	<b>35.87</b>	34.42	<b>36.98</b>	35.35
67	<b>30.10</b>	<b>31.91</b>	31.67	<b>33.19</b>	32.18
68	<b>27.85</b>	<b>28.00</b>	27.51	<b>29.23</b>	28.04
69	<b>23.69</b>	<b>23.29</b>	22.33	<b>23.52</b>	22.84
70	<b>20.48</b>	<b>20.93</b>	19.55	<b>20.77</b>	20.00
71	<b>21.14</b>	<b>21.13</b>	20.07	<b>21.07</b>	20.41
72	<b>23.53</b>	<b>23.08</b>	22.12	<b>23.48</b>	22.66
73	<b>31.39</b>	<b>30.79</b>	30.70	<b>31.99</b>	30.99
74	<b>24.34</b>	<b>23.75</b>	22.94	<b>24.45</b>	23.63
75	<b>26.44</b>	<b>26.23</b>	25.00	<b>26.52</b>	25.71
76	<b>30.45</b>	<b>31.13</b>	29.21	<b>30.95</b>	29.88
77	<b>21.69</b>	<b>22.43</b>	19.08	<b>20.86</b>	19.96
78	<b>38.31</b>	<b>39.00</b>	36.67	<b>38.57</b>	36.42
79	<b>33.84</b>	<b>33.95</b>	31.60	<b>33.34</b>	32.00

**Table S8.** List of Ct values from testing of the Delta variant in RNA extracted from nasopharyngeal swab and throat swab samples. Ct values were obtained using the multiplex Delta assay targeting the T478K and P681R mutations. Ct values provided by Alberta Precision Laboratories (APL) were from RT-PCR analysis of the E gene.

Sample number	Specimen source	T478K Ct	P681R Ct	E gene Ct
		This study		Provided by APL
1	COVID Assessment Site	20.31	20.83	21.92
2	COVID Assessment Site	18.61	19.95	19.61
3	COVID Assessment Site	20.82	21.73	21.49
4	COVID Assessment Site	24.55	25.17	23.49
5	Nasopharyngeal Swab	18.96	19.21	20.09
6	COVID Assessment Site	17.10	17.47	18.76
7	COVID Assessment Site	21.34	21.61	22.97
8	COVID Assessment Site	19.09	22.02	18.84
9	Nasopharyngeal Swab	31.67	33.07	31.18
10	COVID Assessment Site	17.70	20.59	17.31
11	Nasopharyngeal Swab	16.49	18.64	16.13
12	COVID Assessment Site	27.88	31.09	27.51
13	Throat	23.33	24.17	24.89
14	Throat	27.52	29.16	27.67
15	Throat	24.26	25.28	24.96
16	Nasopharyngeal Swab	28.36	28.99	30.59
17	Nasopharyngeal Swab	18.50	19.26	20.03
18	Nasopharyngeal Swab	21.42	24.10	22.89
19	Nasopharyngeal Swab	17.24	19.81	16.47
20	Nasopharyngeal Swab	31.28	31.10	31.71
21	Throat	26.74	27.61	29.99
22	Throat	25.59	26.21	28.36
23	Nasopharyngeal Swab	26.12	28.56	26.97
24	Throat	32.37	34.62	33.05
25	COVID Assessment Site	14.78	15.76	16.68
26	COVID Assessment Site	29.23	30.05	32.87
27	COVID Assessment Site	17.21	18.31	20.35
28	Nasopharyngeal Swab	17.92	18.75	21.68
29	Nasopharyngeal Swab	22.76	23.61	24.79
30	COVID Assessment Site	21.83	22.46	22.87
31	Throat	30.09	30.91	30.27
32	COVID Assessment Site	23.33	25.14	24.76
33	Nasopharyngeal Swab	17.44	18.58	20.87
34	Nasopharyngeal Swab	16.66	19.2	18.55
35	Nasopharyngeal Swab	35.75	36.93	34.24
36	Nasopharyngeal Swab	19.03	20.51	20.81
37	Nasopharyngeal Swab	27.56	30.34	25.75
38	Nasopharyngeal Swab	17.55	16.98	18.71
39	Nasopharyngeal Swab	17.34	18.66	19.19
40	COVID Assessment Site	17.17	18.88	20.36
41	COVID Assessment Site	20.51	21.64	23.78
42	Nasopharyngeal Swab	16.17	17.80	16.84
43	Nasopharyngeal Swab	17.69	19.13	17.69

44	Nasopharyngeal Swab	18.93	20.16	21.11
45	Nasopharyngeal Swab	16.54	18.73	17.82
46	Nasopharyngeal Swab	28.43	27.13	27.62
47	Nasopharyngeal Swab	29.25	30.24	26.25
48	Nasopharyngeal Swab	27.21	28.29	27.60
49	Nasopharyngeal Swab	18.79	19.73	20.57
50	Throat	21.84	24.35	26.36
51	Nasopharyngeal Swab	25.91	27.78	24.57
52	Nasopharyngeal Swab	24.81	24.56	24.79
53	COVID Assessment Site	27.01	29.06	27.04
54	Throat	23.23	23.19	25.24
55	Nasopharyngeal Swab	18.26	20.04	17.71
56	Nasopharyngeal Swab	20.87	24.65	24.12
57	Nasopharyngeal Swab	15.94	17.75	16.19
58	Nasopharyngeal Swab	29.28	30.07	29.50
59	Nasopharyngeal Swab	28.56	30.50	26.69
60	Throat	19.82	22.45	21.62
61	Nasopharyngeal Swab	22.27	23.23	21.78
62	Nasopharyngeal Swab	21.65	22.21	22.84
63	Nasopharyngeal Swab	17.56	18.93	19.35
64	Nasopharyngeal Swab	14.81	15.72	15.00
65	Nasopharyngeal Swab	18.54	19.77	19.44
66	Nasopharyngeal Swab	15.56	16.44	16.49
67	Nasopharyngeal Swab	19.32	21.36	18.02
68	Throat	23.58	24.36	25.07
69	Nasopharyngeal Swab	16.99	18.65	17.71
70	Throat	28.21	28.71	30.60
71	Throat	24.87	26.66	26.37
72	Nasopharyngeal Swab	16.37	16.00	17.98
73	Nasopharyngeal Swab	15.11	16.92	14.97
74	Nasopharyngeal Swab	15.76	17.03	16.66
75	Throat	25.45	26.05	26.20
76	COVID Assessment Site	25.71	26.70	26.98
77	Nasopharyngeal Swab	31.98	32.06	34.58
78	Nasopharyngeal Swab	33.55	33.60	33.10
79	Nasopharyngeal Swab	29.09	30.46	29.58
80	Nasopharyngeal Swab	31.64	32.08	31.44
81	Nasopharyngeal Swab	20.55	20.90	21.25
82	Nasopharyngeal Swab	22.97	23.66	22.40
83	Nasopharyngeal Swab	28.96	29.90	28.35
84	Nasopharyngeal Swab	18.45	18.67	18.62
85	Nasopharyngeal Swab	23.51	23.81	23.51
86	Nasopharyngeal Swab	16.83	17.26	16.66
87	Nasopharyngeal Swab	17.17	17.64	17.01
88	COVID Assessment Site	19.78	21.57	20.50

89	COVID Assessment Site	30.77	28.96	32.43
90	Nasopharyngeal Swab	21.74	23.18	20.37
91	Nasopharyngeal Swab	31.08	31.68	29.83
92	Nasopharyngeal Swab	17.24	17.49	16.63
93	Nasopharyngeal Swab	30.73	30.91	30.67
94	Nasopharyngeal	24.62	24.81	22.84
95	Nasopharyngeal Swab	22.09	23.61	19.43
96	COVID Assessment Site	18.16	19.20	17.55
97	COVID Assessment Site	30.84	31.56	31.93
98	Throat	22.94	22.75	24.37
99	COVID Assessment Site	17.87	17.55	18.51
100	Nasopharyngeal Swab	17.39	17.78	18.45

<sup>a</sup>All the samples in this table have been confirmed to be Delta variant positive by the Alberta Precision Laboratories using genome sequencing.

**Table S9.** Detection of the Alpha, Beta, and Gamma variants in wastewater samples from Calgary and Edmonton, Alberta, Canada

<b>Sample (sampling date)</b>	<b>Total SARS-CoV-2 RNA (N1 gene) (copies/100 mL)</b>	<b>Alpha (HV69/70 deletion) (copies/100 mL)</b>	<b>Beta (K417N) (copies/100 mL)</b>	<b>Gamma (K417T) (copies/100 mL)</b>
<b>Calgary</b>				
05/16/21	26153	862	N/A	14
05/20/21	23375	761	N/A	N/A
05/23/21	4623	310	N/A	N/A
05/27/21	9227	440	N/A	N/A
05/30/21	9404	235	N/A	N/A
06/03/21	15789	218	N/A	6
06/06/21	7180	94	14	N/A
06/10/21	5074	102	N/A	N/A
06/13/21	13215	82	3	14
06/15/21	5836	122	N/A	N/A
06/20/21	5112	14	N/A	67
06/22/21	3138	24	N/A	26
<b>Edmonton</b>				
05/16/21	9787	120	N/A	N/A
05/20/21	12432	142	N/A	69
05/23/21	8112	366	2	3
05/27/21	1559	18	N/A	63
05/30/21	1840	41	N/A	N/A
06/03/21	3212	54	N/A	N/A
06/06/21	2847	52	N/A	N/A
06/10/21	733	147	2	N/A
06/13/21	1994	N/A	N/A	60
06/15/21	1556	21	2	5
06/20/21	558	5	28	4
06/22/21	520	5	N/A	N/A

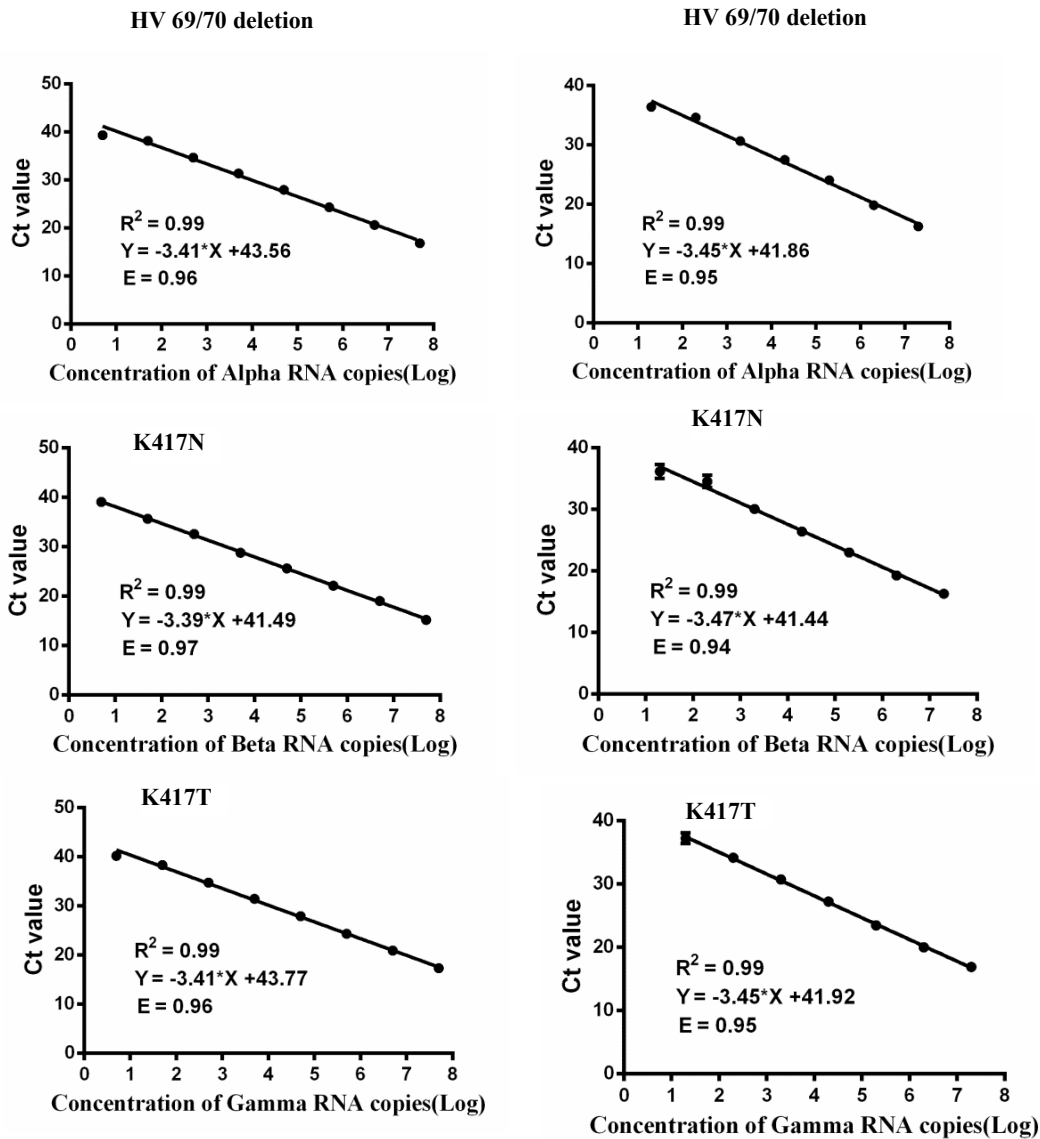


Figure S1. Standard curves of targets HV69/70 deletion and K417N and K417T mutations using singleplex RT-qPCR assays (left figures) and using the ABG multiplex RT-qPCR assay (right figures). The log values of the copies of 10-time diluted pure SARS-CoV-2 variant RNA are plotted against the corresponding Ct values. E represents PCR efficiency which was calculated using the equation:  $E = -1 + 10^{(-1/\text{slope})}$ , where slope refers to the slope of the standard curve. This standard curve was used to quantify the amounts of SARS-CoV-2 variant RNA in the samples.



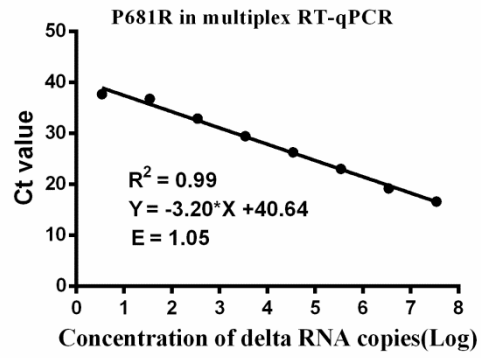
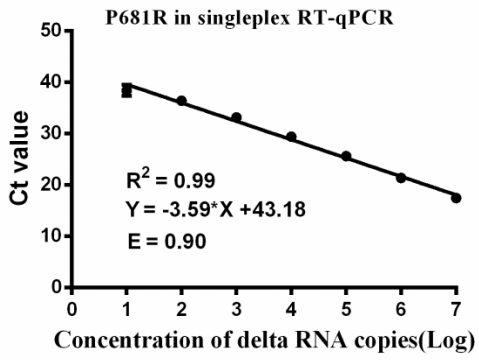
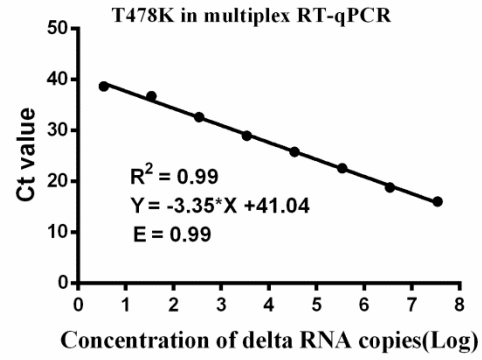
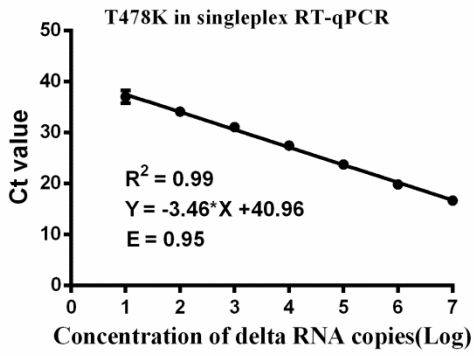


Figure S2. Standard curves of targets T478K and P681R using singleplex RT-qPCR assays (left figures) and using the Delta multiplex RT-qPCR assay (right figures). The log values of the copies of 10-time diluted pure SARS-CoV-2 variant RNA are plotted against the corresponding Ct values. E represents PCR efficiency. This standard curve was used to quantify the amounts of SARS-CoV-2 variant RNA in the samples.

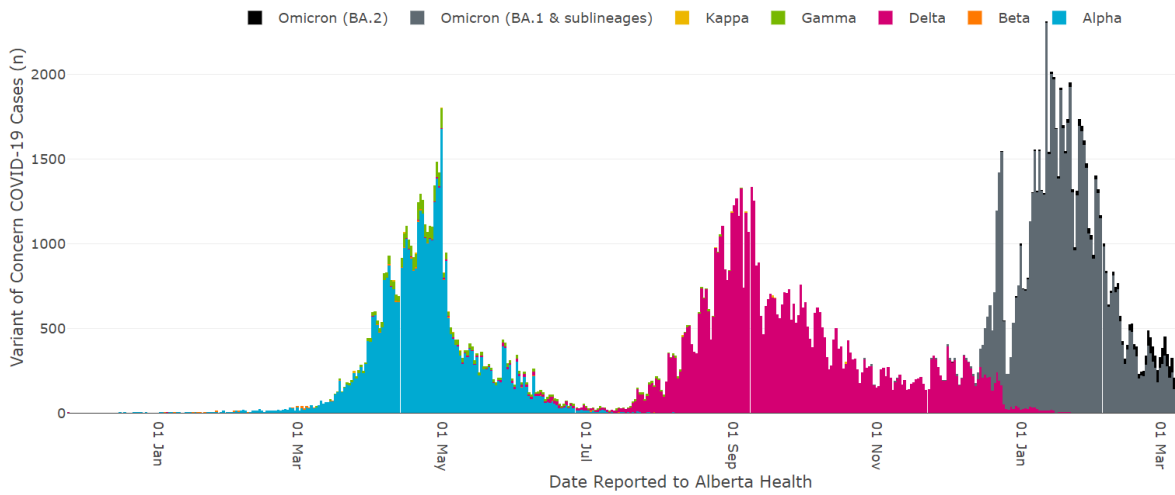


Figure S3. Variants of concern COVID-19 cases in Alberta by day reported by Alberta Health Services (AHS) (<https://www.alberta.ca/stats/covid-19-alberta-statistics.htm#variants-of-concern>)