

**Supplementary information to:**

**SARS-CoV-2 N gene dropout and N gene Ct value shift as indicator for the presence of**

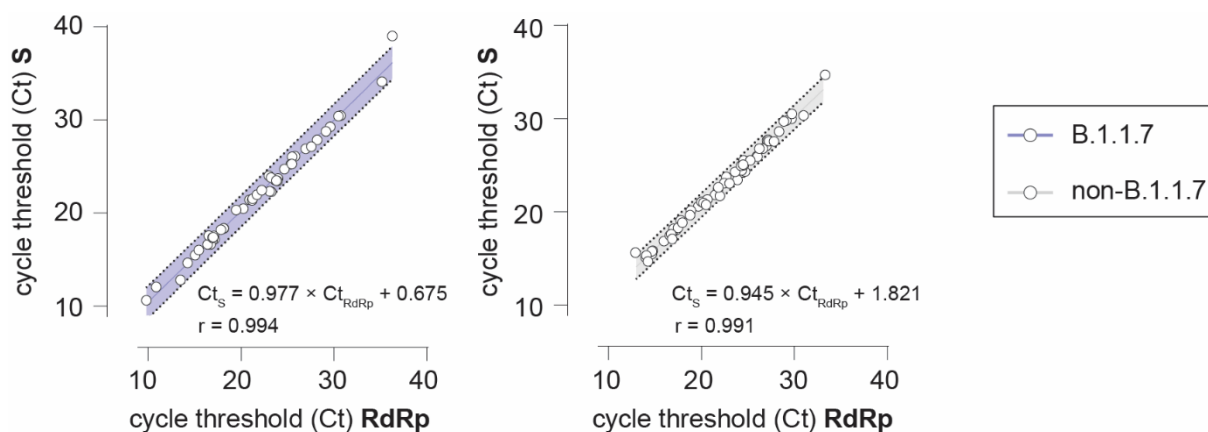
**B.1.1.7 lineage in a commercial multiplex PCR assay**

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	1			12
	M	S	D	T
patient 49 (non-B.1.1.7)	ATG	TCT	GAT	AAT
pCRII-TOPO_N-gene_D3	ATG	TCT	GAT	AAT
patient 1 (B.1.1.7)	ATG	TCT	CTA	AAT
pCRII-TOPO_N-gene_3L	ATG	TCT	CTA	AAT
	M	S	L	T

**Supplement figure 1:** Nucleotide sequence alignment (MUSCLE) of N gene sequences from two exemplary patient samples (non-B.1.1.7 and B.1.1.7, respectively) and pCRII-TOPO\_N-gene\_D3 and \_3L plasmids. Only the region containing the D3L mutation is shown (nucleotide position 1-12), with D3 highlighted in green (non-B.1.1.7) and 3L (B.1.1.7) highlighted in red. Translated amino acid sequences are shown above and below the nucleotide sequences. N gene sequences from the patient samples were extracted from whole-genome sequences.



**Supplement figure 2:** Linear regression of Ct values of S gene and RdRp gene of B.1.1.7 (left panel, blue line and 99% prediction band) and non-B.1.1.7 (right panel, gray line and 99% prediction band) positive patient specimens.

**Supplement table 1:** Included samples (n = 48) with Ct value shifts of  $\Delta N/RdRp$  and  $\Delta N/S$  in the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay with the corresponding results of whole genome sequencing or variant-specific PCR.

Patient #	Allplex™ SARS-CoV-2/FluA/FluB/RSV			$\Delta N/RdRp$	$\Delta N/S$	Lineage / variant PCR*
	S	RdRp	N			
1	26.10	25.80	32.80	7.00	6.70	B.1.1.7
2	17.54	17.09	24.31	7.22	6.77	B.1.1.7
3	26.04	25.48	33.16	7.68	7.12	B.1.1.7
4	22.27	23.26	31.47	8.21	9.20	B.1.1.7
5	18.33	18.13	25.44	7.31	7.11	B.1.1.7
6	26.90	26.96	37.23	10.27	10.33	B.1.1.7
7	21.42	20.91	27.75	6.84	6.33	B.1.1.7
8	18.35	18.10	24.55	6.45	6.20	B.1.1.7
9	22.32	23.07	30.69	7.62	8.37	B.1.1.7
10	12.79	13.47	21.56	8.09	8.77	B.1.1.7
11	15.48	15.05	24.39	9.34	8.91	B.1.1.7
12	17.19	17.15	24.85	7.70	7.66	B.1.1.7
13	29.25	29.59	-	-	-	N501Y+, Del. H69/V70+
14	27.83	28.19	37.48	9.29	9.65	B.1.1.7
15	17.03	16.90	25.92	9.02	8.89	B.1.1.7
16	25.23	25.36	33.51	8.15	8.28	B.1.1.7
17	21.35	21.19	28.51	7.32	7.16	N501Y+, Del. H69/V70+
18	27.14	27.55	35.38	7.83	8.24	N501Y+, Del. H69/V70+
19	23.53	23.66	30.65	6.99	7.12	B.1.1.7
20	14.63	14.23	22.40	8.17	7.77	B.1.1.7
21	20.46	20.25	28.61	8.36	8.15	B.1.1.7
22	10.63	9.80	19.90	10.10	9.27	B.1.1.7
23	24.71	24.65	31.86	7.21	7.15	B.1.1.7
24	28.75	29.14	-	-	-	B.1.1.7
25	23.70	23.97	31.78	7.81	8.08	B.1.1.7
26	16.61	16.71	24.28	7.57	7.67	N501Y+, Del. H69/V70+
27	18.20	17.83	25.86	8.03	7.66	N501Y+, Del. H69/V70+
28	21.54	21.23	29.46	8.23	7.92	N501Y+, Del. H69/V70+
29	16.63	16.39	25.55	9.16	8.92	N501Y+, Del. H69/V70+
30	24.00	23.00	28.88	5.88	4.88	N501Y+, Del. H69/V70+
31	20.50	20.32	27.67	7.35	7.17	N501Y+, Del. H69/V70+
32	12.07	10.85	19.89	9.04	7.82	N501Y+, Del. H69/V70+
33	20.33	19.46	28.30	8.84	7.97	N501Y+, Del. H69/V70+
34	17.57	16.50	25.29	8.79	7.72	N501Y+, Del. H69/V70+
35	23.81	23.23	31.61	8.38	7.80	N501Y+, Del. H69/V70+
36	18.43	18.04	26.31	8.27	7.88	B.1.1.7
37	30.45	30.70	-	-	-	N501Y+, Del. H69/V70+
38	30.40	30.48	-	-	-	N501Y+, Del. H69/V70+
39	34.10	35.15	-	-	-	N501Y+, Del. H69/V70+
40	16.02	15.45	23.73	8.28	7.71	B.1.1.7
41	21.97	21.69	28.61	6.92	6.64	B.1.1.7
42	17.35	16.91	23.66	6.75	6.31	B.1.1.7
43	23.42	23.92	30.73	6.81	7.31	B.1.1.7
44	23.49	23.79	31.06	7.27	7.57	B.1.1.7
45	39.01	36.29	-	-	-	B.1.1.7
46	17.41	16.98	25.59	8.61	8.18	B.1.1.7
47	22.46	22.23	28.52	6.29	6.06	B.1.1.7
48	25.25	25.47	32.25	6.78	7.00	B.1.1.7

\* N501Y+, Del. H69/V70+ indicate presence of lineage B.1.1.7.

**Supplement table 2:** Included samples (n = 58) with no Ct value shifts of  $\Delta N/RdRp$  and  $\Delta N/S$  in the Allplex™ SARS-CoV-2/FluA/FluB/RSV assay with the corresponding results of whole genome sequencing or variant-specific PCR.

Patient #	Allplex™ SARS-CoV-2/FluA/FluB/RSV			$\Delta N/RdRp$	$\Delta N/S$	Lineage / variant PCR
	S	RdRp	N			
49	15.81	14.76	13.62	1.14	2.19	B.1.1.317
50	16.85	15.97	14.79	1.18	2.06	B.1.1.317
51	30.01	29.72	27.62	2.10	2.39	N501Y+, E484K+
52	18.04	17.28	16.94	0.34	1.10	N501Y-, Del. H69/V70-
53	24.24	24.37	23.87	0.50	0.37	N501Y-, Del. H69/V70-
54	24.32	24.68	23.87	0.81	0.45	N501Y-, Del. H69/V70-
55	15.46	14.67	14.47	0.20	0.99	N501Y-, Del. H69/V70-
56	15.77	14.67	14.33	0.34	1.44	N501Y-, Del. H69/V70-
57	17.83	16.91	15.00	1.91	2.83	N501Y-, Del. H69/V70-
58	24.45	24.34	22.52	1.82	1.93	N501Y-, Del. H69/V70-
59	17.44	16.59	15.95	0.64	1.49	N501Y-, Del. H69/V70-
60	25.98	25.97	25.22	0.75	0.76	N501Y-, Del. H69/V70-
61	29.81	29.14	27.36	1.78	2.45	N501Y-, Del. H69/V70-
62	27.07	26.99	25.94	1.05	1.13	N501Y-, Del. H69/V70-
63	28.62	28.35	26.22	2.13	2.40	N501Y-, Del. H69/V70-
64	18.17	16.99	16.11	0.88	2.06	N501Y-, Del. H69/V70-
65	20.57	19.65	18.50	1.15	2.07	N501Y-, Del. H69/V70-
66	15.61	12.88	12.27	0.61	3.34	N501Y-, Del. H69/V70-
67	22.45	21.80	21.14	0.66	1.31	N501Y-, Del. H69/V70-
68	23.74	22.68	21.35	1.33	2.39	N501Y-, Del. H69/V70-
69	21.06	20.01	18.79	1.22	2.27	N501Y-, Del. H69/V70-
70	27.68	27.07	26.11	0.96	1.57	N501Y-, Del. H69/V70-
71	20.12	18.87	17.69	1.18	2.43	N501Y-, Del. H69/V70-
72	15.09	13.98	13.52	0.46	1.57	N501Y-, Del. H69/V70-
73	25.91	26.03	23.74	2.29	2.17	N501Y-, Del. H69/V70-
74	19.64	18.76	18.10	0.66	1.54	N501Y-, Del. H69/V70-
75	18.28	17.49	16.77	0.72	1.51	N501Y-, Del. H69/V70-
76	17.59	16.73	16.09	0.64	1.50	N501Y-, Del. H69/V70-
77	19.08	17.77	16.12	1.65	2.96	N501Y-, Del. H69/V70-
78	23.09	22.45	21.24	1.21	1.85	N501Y-, Del. H69/V70-
79	18.83	17.95	16.56	1.39	2.27	N501Y-, Del. H69/V70-
80	27.74	27.18	26.60	0.58	1.14	N501Y-, Del. H69/V70-
81	15.29	14.08	13.34	0.74	1.95	N501Y-, Del. H69/V70-
82	14.68	14.24	12.99	1.25	1.69	N501Y-, Del. H69/V70-
83	26.78	26.60	25.85	0.75	0.93	N501Y-, Del. H69/V70-
84	17.07	16.86	16.25	0.61	0.82	N501Y-, Del. H69/V70-
85	25.51	24.55	23.89	0.66	1.62	N501Y-, Del. H69/V70-
86	25.01	24.58	23.20	1.38	1.81	N501Y-, Del. H69/V70-
87	23.42	23.88	22.09	1.79	1.33	N501Y-, Del. H69/V70-
88	26.78	26.24	25.44	0.80	1.34	N501Y-, Del. H69/V70-
89	27.59	27.28	26.16	1.12	1.43	N501Y-, Del. H69/V70-
90	16.85	15.97	14.76	1.21	2.09	N501Y-, Del. H69/V70-
91	34.70	33.32	31.63	1.69	3.07	N501Y-, Del. H69/V70-
92	21.83	21.48	19.50	1.98	2.33	N501Y-, Del. H69/V70-
93	24.28	23.63	22.82	0.81	1.46	N501Y-, Del. H69/V70-
94	21.34	20.65	19.76	0.89	1.58	N501Y-, Del. H69/V70-
95	23.06	23.01	21.90	1.11	1.16	N501Y-, Del. H69/V70-
96	27.57	27.82	26.53	1.29	1.04	N501Y-, Del. H69/V70-
97	21.71	21.98	20.94	1.04	0.77	N501Y-, Del. H69/V70-
98	29.71	28.87	27.83	1.04	1.88	N501Y-, Del. H69/V70-
99	20.96	20.20	19.30	0.90	1.66	N501Y-, Del. H69/V70-
100	24.94	24.25	23.36	0.89	1.58	N501Y-, Del. H69/V70-

**Supplement table 2 continued:**

101	30.33	30.99	28.11	2.88	2.22	N501Y-, Del. H69/V70-
102	30.52	29.73	28.49	1.24	2.03	N501Y-, Del. H69/V70-
103	25.52	25.26	24.44	0.82	1.08	N501Y-, Del. H69/V70-
104	22.63	21.81	19.75	2.06	2.88	N501Y-, Del. H69/V70-
105	25.07	24.48	23.45	1.03	1.62	N501Y-, E484K+
106	20.74	20.49	20.39	0.1	0.35	B.1.351

**Supplement table 3:** The Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay discriminates SARS-CoV-2 N-gene 3L from D3 plasmids, while the Allplex™ 2019-nCoV Assay does not.

Copy numbers	Allplex™ SARS-CoV-2/FluA/FluB/RSV			Allplex™ 2019-nCoV		
	N gene D3	N gene L3	ΔCt N	N gene D3	N gene L3	ΔCt N
$1.25 \times 10^8$	13.99	24.34	10.1	17.54	18.61	1.07
$1.25 \times 10^7$	19.15	28.72	9.1	22.53	22.74	0.21
$1.25 \times 10^6$	23.68	31.79	8.1	26.27	26.14	0.13
$1.25 \times 10^5$	28.75	36.74	7.1	30.03	29.87	0.19