Supplemental Material

Design of SARS-CoV-2 variant-specific PCR assays considering regional and temporal characteristics

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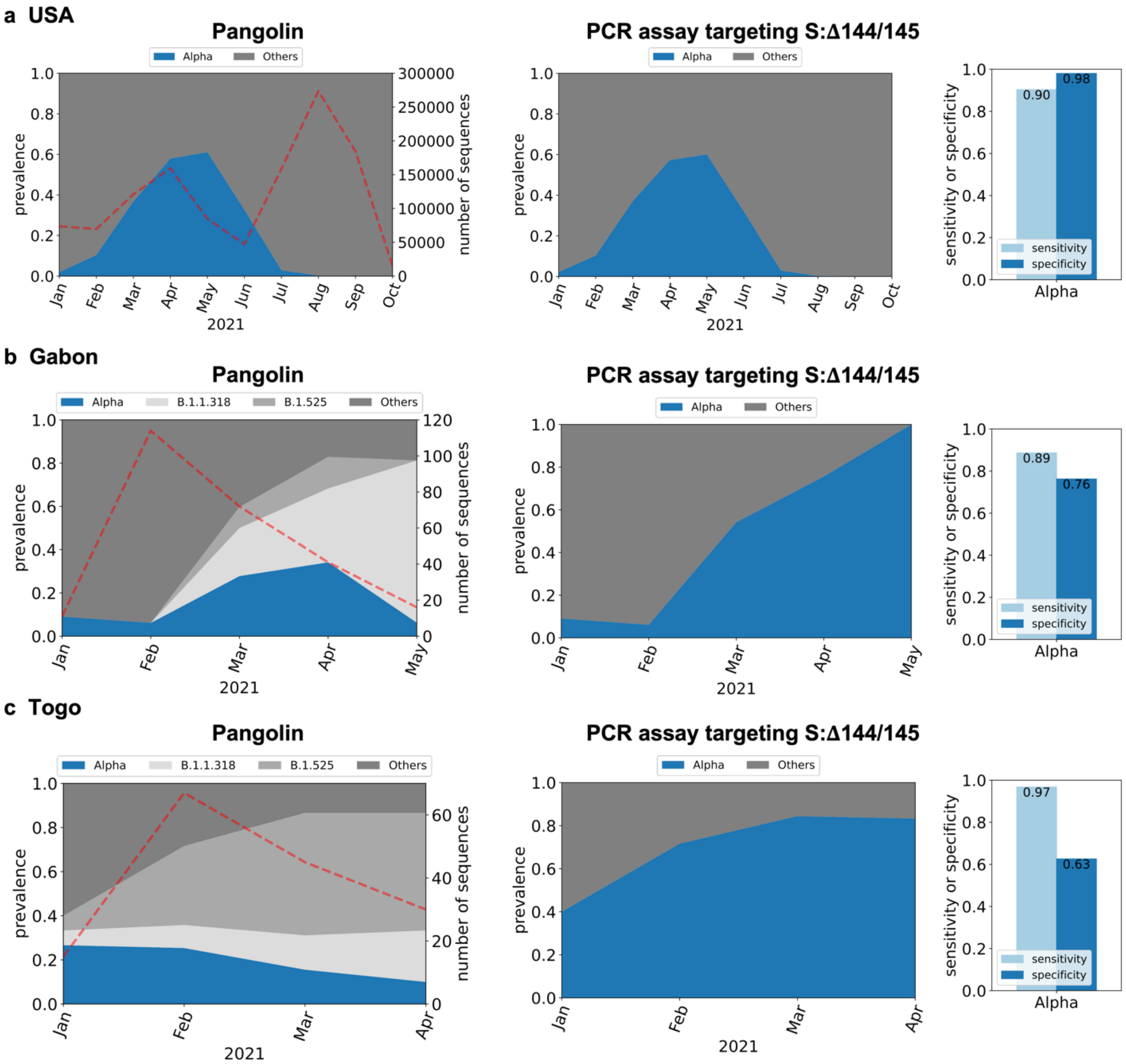
Four tables and four figures are included



SI Fig. 1. *In silico* analysis of PCR assay targeting S:Δ144/145 mutation (1) to detect the Alpha variant for GISAID samples from (**a**) USA (n=1,187,412), (**b**) Gabon (n=254) and (**c**) Togo (n=157). Dotted lines on left figures indicate the number of sequences used for the *in silico* analyses.

SI Table 1. Defining mutations for Alpha, Gamma, and Delta variants with other lineages that cause false positives and false negatives (updated as of September 2021). If the predominance of target mutation in the target variant is less than 90%, lineages are considered to cause false positives in this study. If the predominance of target mutation in the other variants is higher than 10%, lineages are considered false negatives. Lineages with more than 100 sequences reported in GISAID are selectively listed.

Variant	**Defining mutations**	**Analysis using Outbreak.info**
Alpha	S:H69-	B.1.258.19, B.1.258.9, B.1.258.5, B.1.258.7, B.1.258.4, B.1.258.21, B.1.258.11 B.1.620, B.1.415.1, B.1.258.12, B.1.375, B.1.634, B.1.525 (Eta), A.28, B.1.1.298, B.1.258.22, C.36.3.1, C.36.3, B.1.625, B.1.258, B.1.1.189, B.1.1.205, B.1.636 (False positive) Q.2 (False negative)
	S:V70-	
	S:Y144-	AZ.6, B.1.620, AZ.2.1, AZ.2, AZ.4, B.1.525 (Eta), B.1.1.525, B.1.1.318, AZ.5, AZ.3, B.1.637, B.1.630, AV.1, C.1.2, B.1.625, B.1.160.24, AZ.1, B.1.177.80, B.1.1.189, B.1.636, C.38 (False positive)
	S:N501Y	P.1.6, A29, P.1.4, A.27, B.1.621.1, P.1.3, P.1, AP.1, P.1.10, P.1.9, P.1.8, P.1.2, P.1.621, P.1.7, P.1.1, B.1.351.3, B.1.351.2, B.1.351, P.3, C.1.2, B.1.623, B.1.1.189, B.1.153, B.1.597, C.38, B.1.1.205, B.1.636 (False positive) Q.5 (False negative)
	S:A570D	B.1.1.189, C38, B.1.636 (False positive)
	S:D614G	Too many lineages for false positive1)
	S:P681H	P.1.6, B.1.632, AZ.6, AZ.1, B.1.620, B.1.1.519, AZ.4, B.1.631, B.1.575.2, AZ.3, B.1.621.1, AZ.2, AZ.2.1, B.1.1.351, B.1.1.318, B.1.1.526, B.1.621, B.1.415.1, P.3, AZ.5, B.1.628, B.1.575.1, B.1.627, B.1.1.207, B.1.474, P.1.7, AV.1, B.1.469, B.1.575, B.1.243, B.1.468, B.1.400, P.1.1., B.1.189, B.1.1.274, B.1.1.485, B.1.636, B.1.1.163, A.19, B.1.1.161, C.38, B.1.1.46, B.1.1.263 (False positive) Q.4, Q.5, Q.8 (False negative)
	S:T716I	B.1.214.4, B.1.575.2, B.1.214.2, B.1.575.1, C.1.2, B.1.214.3, B.1.575, B.1.1.205, B.1.214, B.1.1.377 (False positive) Q5, Q8 (False negative)
	S:S982A	Q8 (False negative)
	S:D1118H	B.1.620, B.1.1.189 (False positive)
	ORF1a:T1001I	B.1.36.29, AB.1, B.1.636, B.1.1.189, B.1.456 (False positive)
	ORF1a:A1708D	B.1.1.189, B.1.1.205, B.1.636 (False positive)
	ORF1a:I2230T	B.1.1.189, B.1.1.205, B.1.1.226, B.1.636 (False positive)
	ORF1a:S3675-	P.1.6, AZ.6, P.1.10.1, P.1.10.2, P.1.5, AZ.1, B.1.620, B.1.3, B.1.630, P.1.8, B.1.351.2, P.1.9, B.1.619.1, P.1.4, AZ.2, B.1.526, AZ.2.1, P.1.2, B.1.619, B.1.636, P.1.10, B.1.637, AV.1, B.1.1.318, B.1.634, P.1, P.1.7, C.37.1, B.1.1.525, C.37, B.1.351.3, B.1.525, AZ.3, C.1.2, B.1.1.528, AZ.4, B.1.625, B.1.351, B.1.628, P.1.11, P.1.1, AZ.5, B.1.214.2, B.1.470, B.1.1.189, C.38, B.1.1.205,
	ORF1a:G3676-	
	ORF1a:F3677-	

		B.1.241, B.1.214.3, B.1.237, B.1.153, B.1.1.375 (False positive)
	N:D3L	B.1.214.4, B.1.214.2, B.1.214.3, B.1.1.189, B.1.214 (False positive)
	N:R203K	Too many lineages for false positive[2)] Q.5 (False negative)
	N:G204R	Too many lineages for false positive[3)] Q.5, Q.6, Q.7 (False negative)
	N:S235F	B.1.565, B.1.139, B.1.170, C.38, B.1.1.226, B.1.533, B.1.378 (False positive)
	ORF1b:P314L	Too many lineages for false positive[4)] Q.8 (False negative)
	ORF8:Q27*	B.1.1.189, B.1.221.4, B.1.636 (False positive) Q.5, Q.2 (False negative)
	ORF8:R52I	B.1.636, B.1.1.189 (False positive) Q.5, Q.2 (False negative)
	ORF8:Y73C	B.1.1.189, B.1.1.205 (False positive)
Gamma	**S:L18F**	No lineages are discovered for false positives and false negatives
	S:T20N	
	S:P26S	B.1.620, B.1.36.36, B.1.1.420, B.1.177.57 (False positive)
	S:D138Y	B.1.1.333, B.1.561, B.1.1.413, B.1.1.397, B.1.1.354, B.1.1.528, B.1.1.317, B.1.428, B.1.241, B.1.560, B.1.36.24 (False positive) P.1.3 (False negative)
	S:R190S	C.1.2, A.21 (False positive) P.1.2 (False negative)
	S:K417T	P.1.10.2 (False negative)
	S:E484K	AZ.6, B.1.619.1, AZ.2, R.1, B.1.620, AZ.1, B.1.575.2, B.1.626, N.9, AZ.2.1, AZ.3, B.1.1.318, B.1.525, B.1.632, B.1.621.1, B.1.618, AZ.4, B.1.634, B.1.1.523, B.1.619, B.1.621, AZ.5, B.1.1.525, B.1.351.3, AT.1, B.1.351.2, B.1.625, B.1.351, C.1.2, AV.1, B.1.415.1, C.38, B.1.526, B.1.1.207, B.1.1.526 (False positive)
	S:N501Y	A.29, Q.1, Q.7, Q.2, Q.6, Q.3, A.27, B.1.1.7, B.1.621.1, Q.4, AP.1, B.1.621, Q.8, B.1.351.3, B.1.351.2, B.1.351, Q.5, P.3, C.1.2, B.1.623, B.1.1.189, B.1.153, B.1.597, C.38, B.1.1.205, B.1.636 (False positive)
	S:D614G	Too many lineages for false positive[1)]
	S:H655Y	B.1.160.7, B.1.160.33, A.29, B.1.632, A.28, A.27, B.1.630, B.1.1.525, C.1.2, B.1.1.157, B.1.438 (False positive)
	S:T1027I	B.1.619.1, B.1.631, B.1.620, B.1.619, B.1.1.523, B.1.628, B.1.627, B.1.636 (False positive) P.1.1 (False negative)

	S:V1176F	P.4, P.6, P.7, P.2, B.1.1.28, P.3, B.1.324, B.1.177.75 (False positive) P.1.1 (False negative)
	ORF3a:S253P	No lineages are discovered for false positives and false negatives
	ORF1a:S1188L	No lineages are discovered for false positives and false negatives
	ORF1a:K1795Q	B.1.630 (False positive)
	ORF1a:S3675-	AZ.6, AZ.1, B.1.620, Q.7, B.1.630, Q.8, B.1.351.2, B.1.619.1, AZ.2, B.1.526, AZ.2.1, B.1.619, Q.1, Q.4, B.1.636, B.1.637, Q.6, AV.1, B.1.1.318, B.1.634, Q.3, B.1.1.7, C.37.1, B.1.1.525, C.37, B.1.351.3, B.1.525, AZ.3, C.1.2, B.1.1.528, AZ.4, B.1.625, B.1.351, B.1.628, Q.5, AZ.5, B.1.214.2, B.1.470, Q.2, B.1.1.189, C.38, B.1.1.205, B.1.241, B.1.214.3, B.1.237, B.1.153, B.1.1.375 (False positive)
	ORF1a:G3676-	
	ORF1a:F3677-	
	N:P80R	B.1.630 (False positive)
	N:R203K	Too many lineages for false positive5)
	N:G204R	Too many lineages for false positive6)
	ORF1b:P314L	Too many lineages for false positive7)
	ORF1b:E1264D	B.1.1.272, B.1.375, B.1.36.38 (False positive)
	ORF8:E92K	A.23.1, A.29, A (False positive)
Delta	S:T19R	B.1.617.3, B.1.1.374, B.1.623 (False positive) AY.28 (False negative)
	S:E156_	AY.13, AY.5.2, AY.14, AY.21, AY.38, AY.35, AY.15, AY.10, AY.23, AY.19, AY.36, AY.26, AY.16, AY.32, AY.30, AY.1, AY.24, AY.37, AY.17, AY.7.1, AY.33 (False negative)
	S:F157_	
	S:L452R	P.4, C.36.3.1, A.2.5.1, B.1.637, B.1.429, A.27, B.1.427, C.36.3, A.2.5.2, C.16, B.1.617.1, B.1.630, L.3, A.2.5, B.1.1.487, B.1.617.3, B.1.36.35, A.21, B.1.362, B.1.623, C.36, B.1.177.83, B.1.232, B.1.1.354, B.1.459, B.1.575 (False positive) AY.32, AY.28, AY.10 (False negative)
	S:T478K	B.1.1.519, B.1.214.3, B.1.36.1, B.1.623, C.1.2 (False positive) AY.32, AY.28, AY.10 (False negative)
	S:D614G	Too many lineages for false positive1)
	S:P681R	AU.3, AU.2, P.1.8, B.1.617.3, A.23.1, B.1.617.1, B.1.551, B.1.466.2, B.1.1.528, Q.4, B.1.623, B.1.1.25, C.36 (False positive) AY.28 (False negative)
	S:D950N	B.1.621.1, B.1.630, B.1.621, B.1.625, B.1.617.3, B.1.623 (False positive) AY.20, AY.26, AY.16, AY.19, AY.7.2, AY.32, AY.28 (False negative)
	ORF1b:P314L	Too many lineages for false positive7)
	ORF1b:P1000L	A.2.5.1, B.1.634, A.2.5.2, A.27, B.1.1.523, A.25, B.1.628, B.1.623, B.1.459 (False positive) AY.16, AY.28 (False negative)

	M:I82T	AZ.1, AZ.6, B.1.619.1, AZ.4, C.36.3.1, AZ.2, B.1.632, AZ.3, AZ.2.1, B.1.575.2, B.1.525, B.1.1.318, C.36.3, AZ.5, B.1.575.1, B.1.1.523, B.1.415.1, B.1.619, B.1.625, B.1.575, C.1.2, B.1.1.528, C.38, B.1.623 (False positive) AY.32 (False negative)
	N:D63G	B.1.623 (False positive) AY.7.2, AY.23, AY.18 (False negative)
	N:R203M	B.1.617.1, B.1.617.3, B.1.623, B.1.189, B.1.459 (False positive) AY.24, AY.23 (False negative)
	N:D377Y	B.1.1.288, B.1.1.368, B.1.367, B.1617.1, B.1.617.3, B.1.433, B.1.1.229, B.1.36.19, B.1.177.42, B.1.110.3, B.1.177.46, S.1, B.1.258.3, B.1.459, B.1.2, A.2.5.1, B.1.22, B.1.214.3, B.1.623, L.3, B.1.631, AD.2 (False positive) AY.12 (False negative)
	ORF3a:S26L	B.1.617.1, B.1.623 (False positive)
	ORF7a:V82A	B.1.617.3, B.1.617.1, B.1.623, B.1.459 (False positive) AY.7.1, AY.24, AY.23, AY.7.2, AY.27, AY.32, AY.16, AY.17, AY.12 (False negative)
	ORF7a:T120I	B.1.177.18, Q.2, B.1.160.31, B.1.1.58, B.1.623 (False positive) AY.7.1, AY.23, AY.27, AY.16, AY.12 (False negative)

1) https://outbreak.info/situation-reports?pango&muts=S%3AD614G (Last access on September 30th, 2021)
2) https://outbreak.info/situation-reports?pango&muts=N%3AR203K (Last access on September 30th, 2021)
3) https://outbreak.info/situation-reports?pango&muts=N%3AG204R (Last access on September 30th, 2021)
4) https://outbreak.info/situation-reports?pango&muts=ORF1b%3AP314L (Last access on September 30th, 2021)
5) https://outbreak.info/situation-reports?pango&muts=N%3AR203K (Last access on September 30th, 2021)
6) https://outbreak.info/situation-reports?pango&muts=N%3AG204R (Last access on September 30th, 2021)
7) https://outbreak.info/situation-reports?pango&muts=ORF1b%3AP314L (Last access on September 30th, 2021)
8) The up-to-date data will be found via (https://outbreak.info/)

Table 2. Predominance of various sub-lineages in different countries. The countries with higher than 1% predominance for each sub-lineage are reported. The predominance was determined by Outbreak.info. The predominance was determined in Sep. 2021, and the latest predominance should be checked via Outbreak.info.

Sub-lineage	Country	Prevalence (target sequences/total sequences)
A	Too many countries	-
A.19	Côte d'Ivoire	20% (48/236)
	Burkina Faso	12% (48/403)
A.21	Burkina Faso	21% (84/404)
	Mali	4% (3/71)
	Côte d'Ivoire	3% (7/236)
	Central African Republic	2% (1/56)
A.23.1	Rwanda	27% (129/483)
	Uganda	25% (160/628)
	South Sudan	10% (9/88)
	Central African Republic	2% (1/56)
	Democratic Republic of the Congo	2% (11/649)
	Benin	2% (1/65)
	Kenya	1% (48/3341)
	Vietnam	1% (5/357)
	Cambodia	1% (10/992)
A.27	Côte d'Ivoire	10% (24/236)
	Togo	7% (23/343)
	Tunisia	5% (7/129)
	Burkina Faso	5% (21/403)
	Benin	3% (9/263)
	Algeria	2% (1/54)
	Mayotte	2% (12/727)
A.28	Canary Islands	9% (34/358)
	Egypt	2% (21/993)
A.29	Sudan	18% (17/97)
AT.1	Crimea	3% (1/37)
	Russia	1% (113/8195)
AV.1	Namibia	1% (3/262)
	Belarus	1% (1/90)
AY.1	Nepal	5% (12/257)
	Albania	2% (1/42)
	Georgia	1% (3/258)
AY.5.2	Portugal	1% (195/18011)

AY.7.1	Anguilla	27% (3/11)
	Antigua and Barbuda	23% (14/)60
	Armenia	14% (20/140)
	Saint Vincent and the Grenadines	7% (1/14)
	Seychelles	7% (18/256)
	Barbados	6% (5/77)
	Turks and Caicos Islands	6% (1/16)
	Swaziland	6% (7/121)
	Ghana	6% (81/1422)
	Liechtenstein	6% (5/90)
	Denmark	4% (6942/171912)
	Jamaica	3% (5/177)
	Kuwait	2% (7/283)
	Equatorial Guinea	2% (5/205)
	Kosovo	2% (10/483/)
	Lebanon	2% (16/1019)
	Morocco	1% (5/387)
AY.10	Gambia	8% (51/608)
	Senegal	1% (6/513)
	Uzbekistan	1% (1/88)
AY.11	Anguila	9% (1/11)
	Barbados	3% (2/78)
AY.13	U.S. Virgin Islands	5% (18/365)
AY.14	Turks and Caicos Islands	6% (1/16)
AY.16	Kenya	11% (379/3513)
	Kazakhstan	9% (43/460)
	Crimea	8% (3/37)
	Afghanistan	5% (5/94)
	Andorra	4% (1/25)
	South Sudan	3% (3/88)
	Nepal	2% (4/257)
	Mali	1% (1/71)
	Republic of Congo	1% (9/651)
	Oman	1% (11/864)
	India	1% (726/61325)
	Uzbekistan	1% (1/88)
	Gibraltar	1% (17/1648)
AY.17	Nepal	2% (4/257)
AY.19	Swaziland	2% (2/121)

	Kuwait	1% (3/283)
AY.21	Nepal	2% (4/257)
	Lebanon	1% (11/1019)
AY.23	Singapore	54% (4283/7919)
	Brunei	37% (13/35)
	Indonesia	32% (2238/7099)
	Malaysia	14% (454/3252)
	Timor-Leste	9% (31/353)
	Malta	6% (15/256)
	Barbados	4% (3/77)
	Paraguay	3% (10/386)
	Papua New Guinea	2% (8/332)
	Albania	2% (1/42)
	Seychelles	2% (5/256)
	Nepal	2% (4/257)
	Mauritius	2% (4/266)
	Kosovo	1% (7/483)
	Ethiopia	1% (2/140)
	Kazakhstan	1% (5/460)
AY.24	Brunei	43% (15/35)
	Indonesia	10% (710/7099)
	Kosovo	1% (5/483)
AY.26	Bahrain	28% (247/873)
	Mexico	10% (3180/30913)
	Uzbekistan	10% (9/88)
	Anguilla	9% (1/11)
	Bangladesh	3% (91/2791)
	India	3% (1771/61325)
	Albania	2% (1/42)
	Czech Republic	2% (204/9129)
	U.S. Virgin Islands	2% (7/365)
	Costa Rica	2% (24/1273)
	United States	2% (20332/1180345)
	Swaziland	2% (2/121)
	Puerto Rico	2% (52/3232)
	Kuwait	1% (4/283)
	Fiji	1% (7/531)
	Liberia	1% (1/77)
	Malta	1% (3/256)

	Sri Lanka	1% (16/1370)
	Iceland	1% (109/9590)
	Malaysia	1% (33/3252)
AY.30	Australia	29% (9955/34066)
	Thailand	19% (682/3638)
	Cambodia	18% (212/1163)
	Myanmar	9% (7/75)
	Grenada	8% (1/12)
	Kosovo	1% (5/483)
AY.32	South Africa	4% (895/19980)
	Mauritius	3% (9/266)
	Ethiopia	1% (2/140)
	Bahrain	1% (9/873)
AY.33	Maldives	67% (173/258)
	Botswana	44% (461/1036)
	Nepal	27% (70/257)
	Morocco	10% (39/387)
	India	8% (5160/61325)
	Armenia	5% (7/140)
	Bahrain	3% (22/873)
	Ethiopia	2% (3/140)
	Namibia	2% (6/282)
	Costa Rica	2% (27/1273)
	Togo	2% (7/343)
	Switzerland	2% (1370/68952)
	Seychelles	2% (5/256)
	Belgium	2% (932/50135)
	Kenya	2% (63/3513)
	Swaziland	2% (2/121)
	Kuwait	1% (4/283)
	Germany	1% (2750/206790)
	Monaco	1% (1/78)
	Zambia	1% (11/887)
	Uzbekistan	1% (1/88)
	Denmark	1% (1743/171912)
AY.35	British Virgin Islands	3% (1/33)
	Jamaica	1% (2/177)
	Puerto Rico	1% (35/3232)
AY.36	Nigeria	35% (936/2701)

	Burkina Faso	5% (21/404)
	Maldives	4% (10/258)
	Benin	2% (4/262)
AY.37	Liberia	65% (50/77)
	Canada	3% (3990156132/)
	Kuwait	2% (6/283)
	Monaco	1% (1/78)
	Seychelles	1% (3/256)
	U.S. Virgin Islands	1% (4/365)
AY.38	Swaziland	6% (7/121)
	South Africa	5% (941/19980)
	Georgia	2% (4/258)
AZ.1	Cayman Islands	1% (1/73)
AZ.2	Greece	9% (867/9336)
	Togo	1% (5/343)
AZ.2.1	Liechtenstein	12% (13/109)
AZ.4	Ireland	1% (284/32538)
AZ.5	Mauritius	72% (192/266)
B.1.1.7	Too many countries	-
B.1.1.28	Paraguay	11% (42/386)
	Philippines	6% (398/7036)
	brazil	4% (1934/46237)
	uruguay	3% (18/719)
B.1.177.57	Solomon Islands	67% (4/6)
B.1.177.80	Iraq	1% (2/169)
B.1.1.189	Bolivia	2% (1/66)
B.1.1.298	Faroe Islands	2% (1/42)
B.1.1.317	Russia	7% (605/8195)
	Faroe Islands	2% (1/42)
	Estonia	2% (119/6218)
	China	1% (14/1008)
	Belarus	1% (1/87)
	Uzbekistan	1% (1/88)
B.1.1.318	Benin	17% (11/65)
	Gabon	16% (42/267)
	Ghana	13% (176/1337)
	Togo	10% (34/343)
	Nigeria	5% (95/1815)
	Liberia	4% (3/77)

	Guinea	4% (8/214)
	Antigua and Barbuda	2% (1/60)
	Bangladesh	1% (32/2548)
	Cameroon	1% (2/197)
B.1.1.333	Norway	3% (874.27634)
B.1.1.354	Brunei	6% (2/35)
B.1.1.375	Mozambique	11% (63/574)
B.1.1.397	Mongolia	4% (1/28)
	Russia	3% (206/8195)
B.1.1.413	Azerbaijan	7% (1/14)
	Iran	5% (30/552)
B.1.1.420	Cabo Verde	15% (6/39)
	Guinea-Bissau	15% (7/48)
	Senegal	14% (71/513)
	Gambia	2% (15/608)
	Luxembourg	1% (133/12737)
B.1.1.519	Mexico	29% (7752/27035)
	Belize	4% (2/52)
	Martinique	3% (12/387)
	United States	1% (13966/984576)
	Kosovo	1% (1/81)
	Aruba	1% (27/2524)
	Guatemala	1% (7/681)
	Curaçao	1% (7/681)
B.1.1.523	Russia	5% (383/8195)
	Guam	4% (8/196)
	Moldova	1% (1/67)
B.1.1.525	Russia	1% (96/8041)
B.1.36.36	Canada	1% (1639/156132)
B.1.170	Sudan	2% (2/97)
B.1.214.2	Republic of the Congo	22% (48/221)
	Liechtenstein	11% (11/107)
	Democratic Republic of the Congo	2% (13/649)
	Angola	2% (14/860)
	Belgium	2% (737/45463)
B.1.237	Lesotho	6% (1/18)
B.1.241	Costa Rica	1% (17/1273)
B.1.258	Cyprus	54% (72/134)
	Liechtenstein	25% (27/109)

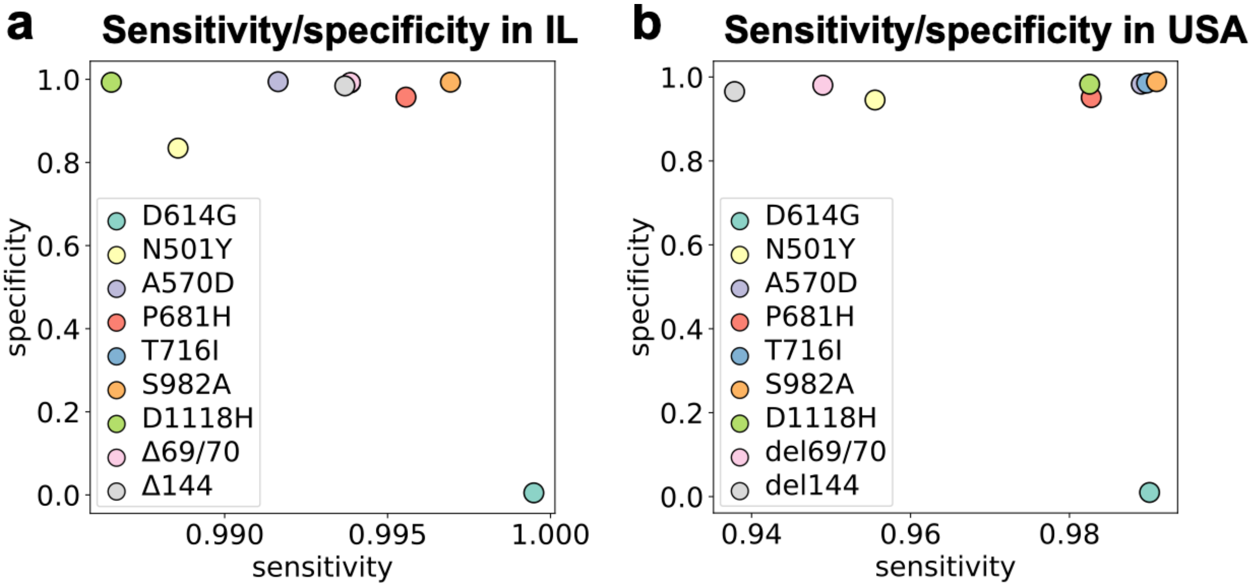
	Faroe Islands	24% (10/42)
	Czech Republic	7% (577/8537)
	Croatia	4% (318/7417)
	Montenegro	4% (8/193)
	Romania	4% (92/2246)
	Bosnia and Herzegovina	3% (7/207)
	Estonia	3% (164/5861)
	Finland	2% (420/18157)
	Slovenia	2% (435/22059)
	Canary Islands	2% (7/358)
	Iceland	2% (183/9603)
	Slovakia	2% (118/6991)
	Macedonia	2% (11/699)
	Poland	2% (318/20532)
	Hungary	1% (6/435)
	Germany	1% (2645/196466)
	Denmark	1% (1998/168597)
	Switzerland	1% (742/66019)
	Sweden	1% (1127/105392)
	Norway	1% (275/26268)
B.1.258.11	Denmark	1% (2468/168597)
B.1.258.17	Slovenia	24% (5313/22059)
	Macedonia	5% (37/699)
	Croatia	2% 167/7417
	Liechtenstein	2% (2/109)
	Bosnia and Herzegovina	1% (3/207)
B.1.324	Djibouti	4% (12/305)
	Northern Mariana Islands	2% (3/152)
B.1.378	The Bahamas	2% (2/133)
B.1.428	Qatar	22% (616/2764)
	Myanmar	1% (1/75)
B.1.438	Iraq	1% (3/220)
B.1.470	Indonesia	7% (485/7099)
	Malaysia	1% (41/3252)
B.1.525	Libya	50% (11/22)
	South Sudan	50% (44/88)
	Mali	44% (31/71)
	Niger	25% (6/24)
	Togo	20% (68/343)

	Côte d'Ivoire	19% (45/236)
	Benin	18% (12/65)
	Nigeria	16% (390/2509)
	Liberia	8% (6/77)
	Burkina Faso	6% (24/403)
	Uganda	6% (37/626)
	Cameroon	6% (11/200)
	Ghana	5% (66/1341)
	Gabon	5% (13/267)
	Malta	4% (11/253)
	Guinea	3% (6/227)
	Kuwait	2% (6/262)
	Senegal	2% (9/491)
	Central African Republic	2% (1/56)
	Moldova	1% (1/67)
	Canada	1% (1828/141562)
	Angola	1% (11/866)
	Belarus	1% (1/90)
B.1.533	Saudi Arabia	1% (11/1093)
B.1.561	Guam	1% (2/196)
B.1.597	Algeria	7% (4/54)
	Tunisia	2% (3/128)
	Morocco	2% (8/387)
B.1.621.1	British Virgin Islands	64% (21/33)
	Dominican Republic	19% (62/325)
	Colombia	6% (226/3839)
	Haiti	5% (5/95)
	Ecuador	2% (51/2202)
	U.S Virgin Islands	1% (5/365)
	Barbados	1% (1/78)
B.1.617.3	Malawi	2% (11/502)
B.1.620	Central African Republic	38% (21/56)
	Republic of the Congo	24% (54/226)
	South Korea	3% (390/13629)
	Cameroon	3% (6/200)
	Gabon	1% (3/267)
B.1.625	Colombia	6% (203/3544)
	Dominican Republic	2% (5/325)
	Venezuela	1% (2/170)

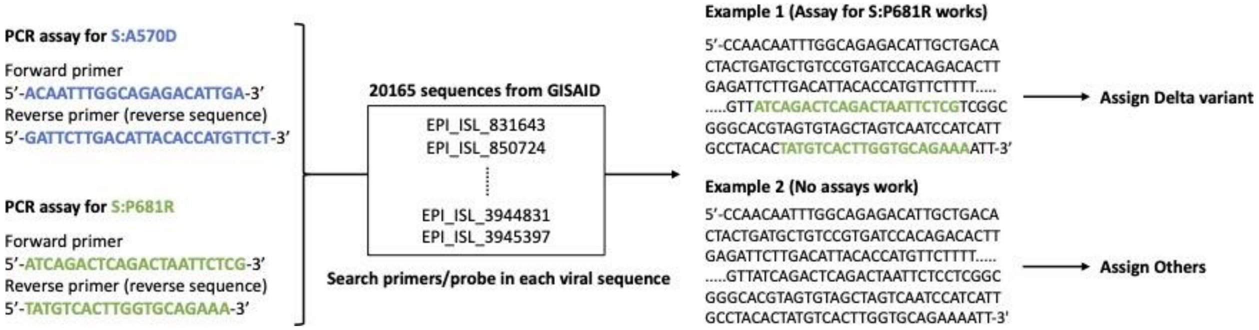
B.1.630	Dominican Republic	8% (26/325)
	Turks and Caicos Islands	6% (1/16)
B.1.634	Honduras	2% (2/116)
	Cayman Islands	1% (1/73)
B.1.636	Honduras	6% (7/116)
B.1.637	Grenada	8% (1/12)
	Dominican Republic	7% (22/325)
	Puerto Rico	4% (133/2993)
	Suriname	3% (16/627)
	Cayman Islands	1% (1/73)
	Jamaica	1% (2/177)
	United States	1% (12319/1114605)
C.1.2	Swaziland	5% (6/121)
C.36.3	Egypt	10% (97/993)
	Belarus	2% (1/51)
	Saudi Arabia	2% (17/1096)
C.38	Egypt	1% (11/993)
P.1	Trinidad and Tobago	62% (357/579)
	Suriname	55% (342/627)
	Brazil	52% (23130/44649)
	Haiti	48% (46/95)
	French Guiana	48% (396/819)
	Chile	38% (3976/10404)
	Guyana	29% (4/14)
	Bolivia	26% (17/66)
	Montserrat	25% (1/4)
	Uruguay	23% (169/720)
	Paraguay	23% (87/386)
	Colombia	16% (597/3839)
	Argentina	15% (1093/7515)
	Dominican Republic	12% (38/325)
	Ecuador	11% (245/2202)
	Costa Rica	11% (135/1273)
	Canada	10% (14706/145942)
	Venezuela	10% (17/170)
	Malta	9% (24/253)
	Mexico	8% (2431/29445)
	Luxembourg	8% (1000/12752)
	Peru	7% (453/6499)

	Barbados	6% (5/78)
	Antigua and Barbuda	5% (3/60)
	Belize	4% (7/178)
	Belgium	4% (1769/48897)
	Guatemala	4% (24/683)
	Faroe Islands	2% (1/42)
	United States	2% (22726/1144329(
	Curaçao	2% (14/707)
	Taiwan	2% (4/239)
	Aruba	2% (39/2442)
	Spain	2% (1031/68050)
	Puerto Rico	1% (47/3213)
	Montenegro	1% (3/216)
	Cayman Islands	1% (1/73)
	Italy	1% (802/60829)
	Jordan	1% (10/779)
	Portugal	1% (189/17493)
P.1.1	Malta	3% (8/253)
	Italy	3% (1716/60829)
	Suriname	2% (13/627)
	Trinidad and Tobago	1% (6/579)
P.1.2	Argentina	2% (143/7515)
	Brazil	2% (693/44649)
	Haiti	1% (1/95)
P.1.4	Brazil	1% (590/44649)
P.1.7	Peru	7% (454/6499)
	Brazil	4% (1667/44649)
	Haiti	2% (2/95)
	Chile	1% (60/10404)
P.1.10	Haiti	7% (7/95)
	Honduras	2% (2/116)
P.2	Suriname	15% (93/626)
	paraguay	10% (38/386)
	french Guiana	9% (70/819)
	Uruguay	6% (41/719)
	brazil	6% (2601/46237)
	canary Islands	6% (24/432)
	argentina	1% (90/7567)
P.3	Philippines	4% (212/5345)

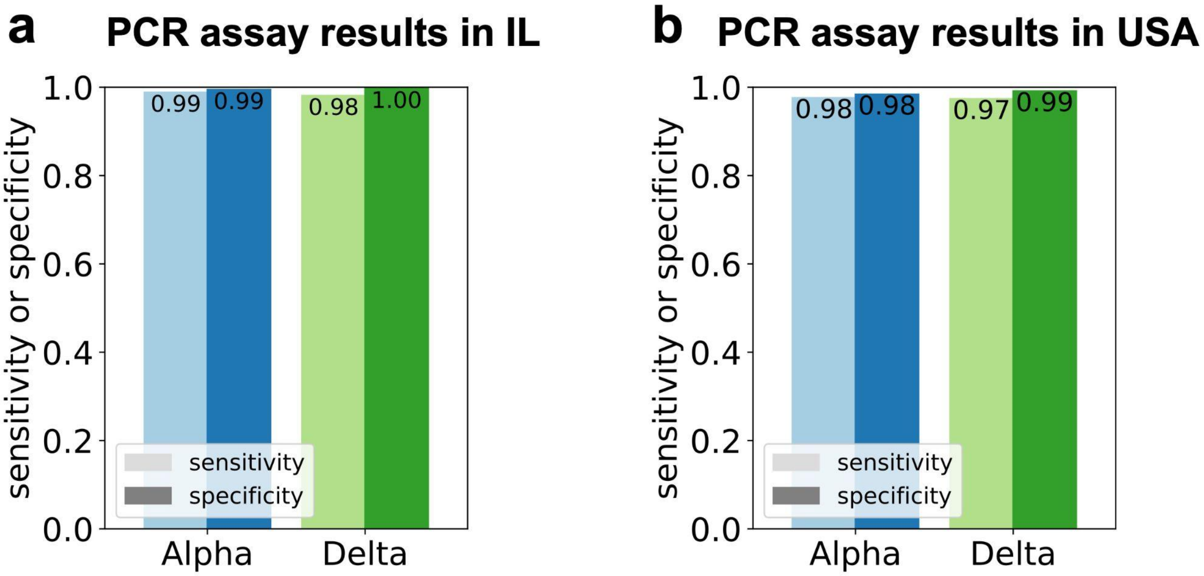
	Guam	2% (3/196)
P.6	Uruguay	39% (283/719)
	Azerbaijan	7% (1/14)
P.7	Uruguay	4% (28/719)
	Paraguay	3% (10/386)
Q.1	Lithuania	25% (4884/19796)
	Belarus	2% (2/87)
	Japan	1% (1650/129656)
	Georgia	1% (3/258)
Q.2	Italy	3% (1796/58744)
Q.4	Georgia	1% (3/256)
	Sudan	1% (1/97)
Q.7	Oman	1% (9/864)
Q.8	Sri Lanka	21% (286/1368)
R.1	Sierra Leone	37% (18/49)
	Japan	6% (7200/129656)
	Guinea	3% (7/223)
	British Virgin Islands	3% (1/33)
	Trindad and Tobago	3% (16/584)
	Liberia	1% (1/77)
	Unknown	1% (18/2845)
	Ecuador	11% (234/2202)
	Haiti	6% (6/95)
	Argentina	6% (455/7567)
	Cameroon	3% (7/202)
	El Salvador	3% (3/104)
	Colombia	2% (72/4116)
	Bolivia	2% (1/66)
	Venezuela	1% (2/171)
	Costa Rica	1% (14/1273)



SI Fig. 2. Sensitivity and specificity, estimated using PRIMES, of determining if GISAID samples from **(a)** IL and **(b)** USA belong to the Alpha variant based on the presence of 9 spike gene mutations that characterize the Alpha variant.



SI Fig. 3. An illustrative example showing the assignment of lineages using PCR assays designed in this study that target mutations S:A570D and S:P681R. In Example 1, the GISAID sequence contains mutation S:P681R and is assigned to belong to the Delta variant. In Example 2, both of the mutations are absent and so the sample is assigned to an auxiliary category 'Others'.



SI Fig. 4. Sensitivity and specificity of the two PCR assays designed in this study, one specific to the Alpha variant and the other specific to the Delta variant, estimated using `PRIMES` in GISAID samples from (a) Illinois and (b) the USA.

79 **SI Table 3.** The checklist from MIQE guidelines and relevant information for this study

Item to check	**Location**
1. Experimental design	
Definition of experimental and control groups	Materials and Methods
Number within each group	Materials and Methods
2. Sample	
Description	Materials and Methods
Volume/mass of sample processed	Materials and Methods
Processing procedure	Materials and Methods
Sample storage conditions and duration	Materials and Methods
3. Nucleic acid extraction	
Procedure and/or instrumentation	Materials and Methods
Name of kit and details of any modifications	Materials and Methods
Contamination assessment (DNA or RNA)	Materials and Methods
Nucleic acid quantification	Materials and Methods
Instrument and method	Materials and Methods
Inhibition testing (C_q dilutions, spike, or other)	Materials and Methods
4. Reverse transcription	
Complete reaction conditions	Materials and Methods
Amount of RNA and reaction volume	Materials and Methods
Reverse transcriptase and concentration	Materials and Methods
Temperature and time	Materials and Methods
Manufacturer of reagents and catalogue numbers	Materials and Methods
5. qPCR target information	
Gene symbol	Materials and Methods, Table 3
Sequence accession number	Materials and Methods, Table 3
Location of amplicon	Materials and Methods, Table 3
Amplicon length	Materials and Methods, Table 3
In silico specificity screen (BLAST, and so on)	Materials and Methods, Table 3
6. qPCR oligonucleotides	
Primer sequences	Materials and Methods, Table 3
Manufacturer of oligonucleotides	Materials and Methods, Table 3

7. qPCR protocol	
Complete reaction conditions	Materials and Methods
Reaction volume and amount of DNA	Materials and Methods
Primer (probe) concentrations	Materials and Methods
Polymerase identity and concentration	Materials and Methods
Buffer/kit identity and manufacturer	Materials and Methods
Manufacturer of plates/tubes and catalog number	Materials and Methods
Complete thermocycling parameters	Materials and Methods
Manufacturer of qPCR instrument	Materials and Methods
8. qPCR validation	
Specificity (gel, sequence, melt, or digest)	Materials and Methods
For SYBR Green I, C_q of the NTC	Materials and Methods
Calibration curves with slope and *y* intercept	Materials and Methods, Fig. 6
PCR efficiency calculated from slope	Materials and Methods
*r*2 of calibration curve	Materials and Methods
Linear dynamic range	Materials and Methods, Fig. 6
C_q variation at LOD	Materials and Methods, Fig. 5
Evidence for LOD	Materials and Methods, Fig. 5
9. Data analysis	
qPCR analysis program (source, version)	Materials and Methods
Method of C_q determination	Materials and Methods
Outlier identification and disposition	Materials and Methods
Results for NTCs	Materials and Methods
Description of normalization method	Materials and Methods
Number and concordance of biological replicates	Materials and Methods
Number and stage of technical replicates	Materials and Methods
Repeatability (intra assay variation)	Materials and Methods
Statistical methods for results significance	Each figure
Software (source, version)	Materials and Methods
Data transparency	Raw data available upon request

SI Table 4. The minimum recommended meta-information on sewage samples (2)

Contents	#1	#2	#3	#4	#5	#6
Sample location type	Street line manhole					
Population served	~1700	~1700	~2400	~2160	~2160	~1100
Combined or separated system	Separated	Separated	Separated	Separated	Separated	Separated
Sample collection type	Time-weighted composite samples					
Sample matrix	Sludge from raw wastewater					
Sample date	01/25/2021	02/01/2021	01/22/2021	02/03/2021	09/19/2021	09/19/2021
Sample time	~11 AM	~11 AM	~10 AM	~10 AM	~10 AM	~10 AM
Sample location	Campus town	Campus town	NE Rantoul	SE Rantoul	SE Rantoul	South Rantoul
Pre-concentration storage temperature	Samples were delivered to the laboratory on ice and processed without freezing on the same day.					
Concentration method and citation	RNA extraction from sludge (3)					
Recovery control name & efficiency	BCoV (0.58%)	BCoV (0.58%)	BCoV (0.74%)	BCoV (2.37%)	BCoV (0.67%)	BCoV (0.71%)
Extraction method & citation	Viral RNA Mini Kit (Quigen, German)					
Amount of sample processed	1785 mL	1855 mL	585 mL	245 mL	1155 mL	862 mL
Extraction blanks results	Signal not detected					
PCR type	qPCR					
SARS-CoV-2 concentrations (gc/L)	2.6×10^3	1.3×10^3	2.3×10^4	9.6×10^3	4.3×10^4	6.0×10^4
Target gene	N1 gene (CDC)					
Endogenous wastewater control name & concentration	PMMoV					
Required MIQE guideline	Summarized in SI Table 3					

Reference

1. Lin Lee W, Imakaev M, Armas F, McElroy KA, Gu X, Duvallet C, Chandra F, Chen H, Leifels M, Mendola S, Floyd-O R, Powell MM, Wilson ST, J Berge KL, J Lim CY, Wu F, Xiao A, Moniz K, Ghaeli N, Matus M, Thompson J, Alm EJ. 2021. Quantitative SARS-CoV-2 Alpha Variant B.1.1.7 Tracking in Wastewater by Allele-Specific RT-qPCR. Cite This Environ Sci Technol Lett 8:675–682.
2. McClary-Gutierrez JS, Aanderud ZT, Al-faliti M, Duvallet C, Gonzalez R, Guzman J, Holm RH, Jahne MA, Kantor RS, Katsivelis P, Kuhn KG, Langan LM, Mansfeldt C, McLellan SL, Grijalva LMM, Murnane KS, Naughton CC, Packman AI, Paraskevopoulos S, Radniecki TS, Roman FA, Shrestha A, Stadler LB, Steele JA, Swalla BM, Vikesland P, Wartell B, Wilusz CJ, Wong JCC, Boehm AB, Halden RU, Bibby K, Vela JD. 2021. Standardizing data reporting in the research community to enhance the utility of open data for SARS-CoV-2 wastewater surveillance. Environ Sci Water Res Technol 7:1545–1551.
3. Wolfe MK, Archana A, Catoe D, Coffman MM, Dorevich S, Graham KE, Kim S, Grijalva LM, Roldan-Hernandez L, Silverman AI, Sinnott-Armstrong N, Vugia DJ, Yu AT, Zambrana W, Wigginton KR, Boehm AB. 2021. Scaling of SARS-CoV-2 RNA in Settled Solids from Multiple Wastewater Treatment Plants to Compare Incidence Rates of Laboratory-Confirmed COVID-19 in Their Sewersheds. Environ Sci Technol Lett 8:398–404.