

La Rosa et al. "Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the Spike protein"

**Study in GISAID on the predictive capacity of specific mutations for the identification of SARS-CoV-2 variants**

**VOC 202012/01 ("UK variant") - GISAID 20I/501Y.V1 - clade GR, lineage B.1.1.7**

Last run:	14.01.2021 h 23.30-23.40				
tot GISAID CoV-2 complete genome seq		371470			
tot GR B.1.1.7 complete genome seq		17798	"y"		
tot VUI202012/01 complete genome seq		17356	"z"		
tot GR B.1.1.7 + VUI202012/01 complete genome seq		17312			

**Number of complete genomes classified as GR B.1.1.7 that display the following combinations of mutations**

H69del	Y145del	N501Y	A570D	n°	n°/y	n°/z
1	1	1	1	17353	97,50%	99,98%
1	1	1	0	17355	97,51%	99,99%
0	1	1	1	17372	97,61%	100,09%
1	0	1	1	17510	98,38%	100,89%
1	1	0	1	17371	97,60%	100,09%
1	1	0	0	17374	97,62%	100,10%
0	0	1	1	17691	99,40%	101,93%
1	0	0	1	17529	98,49%	101,00%
0	1	1	0	17374	97,62%	100,10%
1	0	1	0	17517	98,42%	100,93%
0	1	0	1	17390	97,71%	100,20%
1	0	0	0	17537	98,53%	101,04%
0	1	0	0	17393	97,72%	100,21%
0	0	1	0	17703	99,47%	102,00%
0	0	0	1	17829	100,17%	102,73%
0	0	0	0	17798	100,00%	102,55%

**Number of complete genomes with the following combinations of mutations that are classified as GR B.1.1.7**

H69del	Y145del	N501Y	A570D	n°	GR B.1.1.7	GR B.1.1.7/n°	n°/y	n°/z
1	1	1	1	17355	17355	100,00%	97,51%	99,99%
1	1	1	0	17357	17357	100,00%	97,52%	100,01%
0	1	1	1	17374	17374	100,00%	97,62%	100,10%
1	0	1	1	17512	17512	100,00%	98,39%	100,90%
1	1	0	1	17373	17373	100,00%	97,61%	100,10%
1	1	0	0	17376	17376	100,00%	97,63%	100,12%
0	0	1	1	17693	17693	100,00%	99,41%	101,94%
1	0	0	1	17531	17531	100,00%	98,50%	101,01%
0	1	1	0	17376	17376	100,00%	97,63%	100,12%
1	0	1	0	17519	17519	100,00%	98,43%	100,94%
0	1	0	1	17392	17392	100,00%	97,72%	100,21%
1	0	0	0	24166	17537	72,57%	135,78%	139,24%
0	1	0	0	17908	17395	97,14%	100,62%	103,18%
0	0	1	0	18751	17705	94,42%	105,35%	108,04%
0	0	0	1	17833	17831	99,99%	100,20%	102,75%
0	0	0	0	17798	17798	100,00%	100,00%	102,55%

Y145del corresponds to Y144del

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**VOC 202012/02 ("South Africa") - GISAID 20H/501Y.V2 - clade GR, lineage B.1.351**

Last run:	17.01.2021 h 19.45-23.05								
tot GISAID CoV-2 complete genome seq				379322					
tot GH B.1.351 complete genome seq				533	"y"				
tot 501Y.V2 complete genome seq				474	"z"				
tot GH B.1.351 + 501Y.V2 complete genome seq				471					
<b>Number of complete genomes classified as GH B.1.351 that display the following combinations of mutations</b>									
<b>D80A</b>	<b>E484K</b>	<b>N501Y</b>	<b>L242del</b>	<b>n°</b>			<b>n°/y</b>	<b>n°/z</b>	
1	1	1	1	436			81,80%	91,98%	
1	1	1	0	446			83,68%	94,09%	
0	1	1	1	445			83,49%	93,88%	
1	0	1	1	440			82,55%	92,83%	
1	1	0	1	444			83,30%	93,67%	
1	1	0	0	455			85,37%	95,99%	
0	0	1	1	449			84,24%	94,73%	
1	0	0	1	460			86,30%	97,05%	
0	1	1	0	477			89,49%	100,63%	
1	0	1	0	450			84,43%	94,94%	
0	1	0	1	453			84,99%	95,57%	
1	0	0	0	472			88,56%	99,58%	
0	1	0	0	487			91,37%	102,74%	
0	0	1	0	483			90,62%	101,90%	
0	0	0	1	472			88,56%	99,58%	
0	0	0	0	533			100,00%	112,45%	
<b>Number of complete genomes with the following combinations of mutations that are classified as GH B.1.351</b>									
<b>D80A</b>	<b>E484K</b>	<b>N501Y</b>	<b>L242del</b>	<b>n°</b>	<b>GH B.1.351</b>	<b>GH B.1.351/n°</b>	<b>n°/y</b>	<b>n°/z</b>	
1	1	1	1	439	436	99,32%	82,36%	92,62%	
1	1	1	0	449	446	99,33%	84,24%	94,73%	
0	1	1	1	448	445	99,33%	84,05%	94,51%	
1	0	1	1	443	440	99,32%	83,11%	93,46%	
1	1	0	1	447	444	99,33%	83,86%	94,30%	
1	1	0	0	458	455	99,34%	85,93%	96,62%	
0	0	1	1	452	449	99,34%	84,80%	95,36%	
1	0	0	1	463	460	99,35%	86,87%	97,68%	
0	1	1	0	493	477	96,75%	92,50%	104,01%	
1	0	1	0	453	450	99,34%	84,99%	95,57%	
0	1	0	1	456	453	99,34%	85,55%	96,20%	
1	0	0	0	498	472	94,78%	93,43%	105,06%	
0	1	0	0	761	487	63,99%	142,78%	160,55%	
0	0	1	0	19034	483	2,54%	3571,11%	4015,61%	
0	0	0	1	510	472	92,55%	95,68%	107,59%	
0	0	0	0	533	533	100,00%	100,00%	112,45%	
<b>Y145del</b>	corresponds to Y144del								

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**Confirmation of the discriminatory capacity of the PCR ID 980 in Nextclade (<https://clades.nextstrain.org/>)**

Sequences (full genome or partial Spike gene sequence included in PCR ID 980) used for the study

Clade	GISAID sequence ID	Nextclade	
		Full genome	PCR 980
20I/501Y.V1 (UK variant)	hCoV-19/Denmark/DCGC-23017/2020 EPI ISL 793399 2020-12-28	20I/501Y.V1	20B
	hCoV-19/Portugal/PT2159/2021 EPI ISL 801520 2021-01-02		
	hCoV-19/Norway/7072/2020 EPI ISL 813977 2020-12-21		
	hCoV-19/Netherlands/NH-RIVM-21371/2020 EPI ISL 826536 2020-12-28		
	hCoV-19/England/205390794/2021 EPI ISL 846595 2021-01-02		
20H/501Y.V2 (South African variant)	hCoV-19/South Africa/KRISP-EC-K005341/2020 EPI ISL 678626 2020-11-12	20H/501Y.V2	20H/501Y.V2
	hCoV-19/South Africa/NHLS-UCT-GS-2090-KRISP/2020 EPI ISL 696452 2020-11-19		
	hCoV-19/South Africa/NHLS-UCT-GS-3558-KRISP/2020 EPI ISL 696459 2020-11-20		
	hCoV-19/South Africa/NHLS-UCT-GS-7043-KRISP/2020 EPI ISL 696494 2020-11-24		
	hCoV-19/South Africa/KRISP-K007135/2020 EPI ISL 825130 2020-11-24		
20J/501Y.V3 (Brazilian variant)	hCoV-19/Brazil/AM-20843269RC/2020 EPI ISL 833140 2020-12-23	20J/501Y.V3	20J/501Y.V3
	hCoV-19/Japan/IC-0562/2021 EPI ISL 792681 2021-01-02		
	hCoV-19/Brazil/AM-994/2020 EPI ISL 833174 2020-12-22		
	hCoV-19/Japan/TY7-501/2021 EPI ISL 833366 2021-01		
	hCoV-19/South Korea/KDCA0464/2021 EPI ISL 833249 2021-01-10		
20E.EU1 (Spanish variant)	hCoV-19/Ireland/WW-NVRL-AIIDV1206v1/2020 EPI ISL 768773 2020-09-25	20E (EU1)	20E (EU1)
	hCoV-19/Spain/CT-IBV-97012007/2020 EPI ISL 855493 2020-09-18		
	hCoV-19/Italy/CAM-COTUGNO-11204/2020 EPI ISL 855573 2020-12-21		
	hCoV-19/Denmark/DCGC-32260/2020 EPI ISL 856676 2020-12-07		
	hCoV-19/Belgium/ULG-11309/2021 EPI ISL 856753 2021-01-13		
CAL 20C	hCoV-19/USA/NM-CDC-2-3768096/2021 EPI_ISL_903839 2021-01-02	20C	20C
	hCoV-19/USA/CT-CDC-2-3774026/2020 EPI_ISL_903921 2020-12-03		

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(variant common in California)	hCoV-19/USA/CA-CDC-2-3774040/2020 EPI_ISL_903892 2020-12-31		
	hCoV-19/USA/CA-LACPHL-AF00262/2021 EPI_ISL_905771 2021-01-10		
	hCoV-19/USA/WA-OHSU-9371/2021 EPI_ISL_906034 2021-01-07		
19A (Wuhan strain)	hCoV-19/Italy/FVG-ISS-2249/2020 EPI_ISL_856893 2020-03-01		
	hCoV-19/Ireland/D-20G41967/2020 EPI_ISL_848165 2020-03-26		
	hCoV-19/USA/NY-AECOM_075/2020 EPI_ISL_826638 2020-04-07	19A	19A
	hCoV-19/Italy/LAZ-INMI-SPL1/2020 EPI_ISL_412974 2020-01-29		
	hCoV-19/Italy/LOM-UniMI-L228/2020 EPI_ISL_542215 2020-03-11		

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**Field samples details**

**Clinical samples**

Clinical sample ID	Source swab ID	Sampling date	GeneFinder™ Covid-19 Plus RealAmp Kit		La Rosa et al., 2021 nsp14 region	
			RdRp gene	N gene	Ct	g.c./μL
			Ct	Ct		
swab_1	38846	23/11/2020	21,1	21,2	20,30	1,91E+05
swab_2	41038	28/11/2020	26,0	23,8	20,70	1,46E+05
swab_3	41056	28/11/2020	20,1	20,5	18,87	4,95E+05
swab_4	41057	28/11/2020	19,7	19,1	18,83	5,07E+05
swab_5	48413	31/12/2020	19,9	19,9	19,54	3,17E+05
swab_6	48423	31/12/2020	20,1	20,0	19,14	4,11E+05
swab_7	48432	31/12/2020	18,3	17,7	17,71	1,07E+06
swab_8	48446	31/12/2020	20,6	20,1	19,40	3,46E+05
swab_9	51287	18/01/2021	21,6	19,6	20,24	1,98E+05
swab_10	51289	18/01/2021	23,5	22,7	22,17	5,48E+04
swab_11	51291	18/01/2021	25,8	25,5	25,57	5,69E+03
swab_12	51304	18/01/2021	21,1	19,8	20,85	1,32E+05
swab_13	38850	23/11/2020	29,1	28,9	28,25	9,52E+02
swab_14	38892	23/11/2020	30,8	30,3	30,13	2,72E+02
swab_15	38904	23/11/2020	31,2	30,3	30,76	1,80E+02
swab_16	40983	28/11/2020	24,3	24,7	23,27	2,63E+04
swab_17	48326	31/12/2020	32,4	31,2	31,94	8,15E+01
swab_18	48330	31/12/2020	29,3	28,2	30,06	2,85E+02
swab_19	48458	31/12/2020	30,5	29,8	29,96	3,05E+02
swab_20	48465	31/12/2020	30,7	30,0	29,73	3,57E+02
swab_21	51260	18/01/2021	34,5	31,5	33,33	3,22E+01
swab_22	51267	18/01/2021	33,4	31,7	32,15	7,12E+01
swab_23	51280	18/01/2021	34,4	31,6	32,37	6,14E+01
swab_24	51296	18/01/2021	33,6	31,3	31,34	1,22E+02

Clinical sample ID	Source culture ID	Sampling date	GeneFinder™ Covid-19 Plus RealAmp Kit		La Rosa et al., 2021 nsp14 region	
			RdRp gene	N gene	Ct	g.c./μL
			Ct	Ct		
swab_25	407	24/12/2020	15,6	15,6	13,17	2,21E+07
swab_26	414	24/12/2020	16,5	15,8	14,11	1,18E+07
swab_27	170	25/08/2020	14,4	14,4	10,85	1,03E+08
swab_28	172	25/08/2020	16,1	17,0	15,03	6,37E+06
swab_29	413	24/12/2020	14,3	14,4	10,46	1,34E+08
swab_30	423	30/12/2020	14,6	14,6	11,02	9,24E+07
swab_31	399	24/12/2020	16,2	14,9	13,10	1,74E+07

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<b>Clinical sample ID</b>	<b>GISAID sequence ID</b>	<b>GISAID identification based on WGS</b>	<b>Nextstrain identification based on WGS</b>
swab_25	hCoV-19/Italy/APU-IZSPB 407PT/2020 EPI ISL 794749 2020-11-25	G B.1	20A
swab_26	hCoV-19/Italy/APU-IZSPB 414PT/2020 EPI ISL 794755 2020-11-25	G B.1	20A
swab_27	hCoV-19/Italy/APU-IZSPB-170APT/2020 EPI ISL 649190 2020-08-17	GR B.1.1.316	20B
swab_28	hCoV-19/Italy/APU-IZSPB-172APT/2020 EPI ISL 649191 2020-08-20	GR B.1.1.229	20B
swab_29	hCoV-19/Italy/APU-IZSPB 413PT/2020 EPI ISL 794754 2020-12-16	GV B.1.177	20E EU1
swab_30	hCoV-19/Italy/APU-IZSPB 423PT/2020 EPI ISL 794759 2020-12-22	GV B.1.177	20E EU1
swab_31	hCoV-19/Italy/APU-IZSPB-399PT/2020 EPI ISL 745193 2020-12-21	GR B.1.1.7	20I/501Y.V1

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**Environmental samples**

Sewage sample ID	WWTP ID	Sampling date
<b>Archive samples</b>		
3702	Rome Ostia	26/09/2020
3714	Rome East	05/10/2020
3716	Rome East	14/10/2020
3720	Rome South	12/10/2020
3732	Rome North	26/10/2020
3737	Rome East	04/11/2020
3738	Rome East	04/11/2020
3739	Rome East	13/11/2020
3740	Rome East	13/11/2020
3742	Rome Ostia	11/11/2020
3744	Rome East	19/11/2020
3750	Rome North	11/11/2020
3797	Rome East	17/11/2020
3798	Rome East	17/11/2020
3801	Rome East	02/12/2020
3802	Rome East	02/12/2020
3805	Rome East	14/12/2020
3808	Rome South	12/12/2020
3809	Rome Ostia	11/12/2020
3815	Rome South	26/12/2020
<b>Ad-hoc samples from outbreak locations</b>		
3862	Guardiagrele (CH), Abruzzo	21/01/2021
3863	Guardiagrele (CH), Abruzzo	26/01/2021
3864	Guardiagrele (CH), Abruzzo	26/01/2021
3865	Guardiagrele (CH), Abruzzo	26/01/2021
3866	Guardiagrele (CH), Abruzzo	26/01/2021
3867	Guardiagrele (CH), Abruzzo	26/01/2021
3868	Guardiagrele (CH), Abruzzo	26/01/2021
3869	Guardiagrele (CH), Abruzzo	26/01/2021
3944	Perugia, Umbria	05/02/2021
3945	Perugia, Umbria	05/02/2021
3946	Perugia, Umbria	06/02/2021
3947	Perugia, Umbria	06/02/2021
3948	Perugia, Umbria	08/02/2021
3949	Perugia, Umbria	08/02/2021

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**Primer used for sequencing the PCR fragment of PCR ID 980 (long nested assay)**

<b>Primer ID</b>	<b>Sequence (5'-3')</b>	<b>Positions *</b>	<b>Strand</b>
2321	TTCAACTCAGGACTTGTTCTTACC	21709-21732	forward
2322RevCom	TTGGATGGAAAGTGAGTTCAGA	22036-22015	forward
2323	TTGTTTAGGAAGTCTAATCTCAAACC	22925-22950	forward
2320	GCTGAGAGACATATTCAAAGTGCA	22082-22058	reverse
2325RevCom	GTGTGCTACCGCCTGATAG	22978-22997	reverse
2326	GTGGATCACGGACAGCATC	23300-23282	reverse

\* Reference genome NC45512.2 (Wuhan)



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**Geographic and temporal occurrence of variants detected in clinical samples using PCR ID 980**

Sample	Mutation map	Mutation occurrence (GISAID CoVsurver Mutation Analysis)
swab_1, swab_4, swab_5, swab_6, swab_7, swab_8, swab_9, swab_10, swab_11, swab_12, swab_17, swab_20, swab_29, swab_30	A222V	AA change Spike A222V already occurred 93796 times (23.50% of all samples with Spike sequence) in 58 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/Iran/K1r-29/2020. The aa change most recently occurred in strain hCoV-19/England/SHEF-10CF750/2021, collected in January 2021.
swab_2, swab_3, swab_13, swab_16, swab_23	S477N	AA change Spike S477N already occurred 20280 times (5.57% of all samples with Spike sequence) in 42 countries. The first strain with this aa change, collected in March 2020, was hCoV-19/Australia/VIC2165/2020. The aa change most recently occurred in strain hCoV-19/Singapore/56/2021, collected in January 2021.
swab_14, swab_15, swab_25, swab_27, swab_28	-	///
		AA change Spike A222V already occurred 93796 times (23.50% of all samples with Spike sequence) in 58 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/Iran/K1r-29/2020. The aa change most recently occurred in strain hCoV-19/England/SHEF-10CF750/2021, collected in January 2021.
swab_18	A222V, A262S, P272L	AA change Spike A262S already occurred 4248 times (1.17% of all samples with Spike sequence) in 32 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/South Korea/KCDC2085/2020. The aa change most recently occurred in strain hCoV-19/England/210241211/2021, collected in January 2021.
		AA change Spike P272L already occurred 3181 times (0.87% of all samples with Spike sequence) in 26 countries. The first strain with this aa change, collected in March 2020, was hCoV-19/Australia/QLD1278/2020. The aa change most recently occurred in strain hCoV-19/England/210241211/2021, collected in January 2021.
swab_19	S98F	AA change Spike S98F already occurred 4626 times (1.27% of all samples with Spike sequence) in 41 countries. The first strain with this aa change, collected in January 2020, was hCoV-19/Shanghai/SH0010/2020. The aa change

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		most recently occurred in strain hCoV-19/Belgium/ULG-11273/2021, collected in January 2021.
swab_24	A222V, A411S	AA change Spike A222V already occurred 93796 times (23.50% of all samples with Spike sequence) in 58 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/Iran/K1r-29/2020. The aa change most recently occurred in strain hCoV-19/England/SHEF-10CF750/2021, collected in January 2021.
		AA change Spike A411S already occurred 40 times (0.01% of all samples with Spike sequence) in 9 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/Wuhan/HB-WH2-166/2020. The aa change most recently occurred in strain hCoV-19/England/QEUH-D513EC/2020, collected in December 2020.
swab_25	Swab 25 provided a double electropherogram signal, one of which including a nine amino acid deletion: IHVSGTNGT 68-76del	N74del and T76del. Mutation Spike N74del and T76del removes a potential N-glycosylation site at position 74, which may also affect antigenic and other properties of this strain. In detail, the motif at positions 74-76 changed from NGT (glyco) to --- (no glyco).
swab_26	D215H	AA change Spike D215H already occurred 1155 times (0.32% of all samples with Spike sequence) in 22 countries. The first strain with this aa change, collected in February 2020, was hCoV-19/Hangzhou/ZJU-010/2020. The aa change most recently occurred in strain hCoV-19/USA/NY-Wadsworth-21002030-01/2021, collected in January 2021.
swab_31	H69del, V70del, Y144del, N501Y, A570D	AA change Spike N501Y already occurred 19062 times (5.23% of all samples with Spike sequence) in 45 countries. The first strain with this aa change, collected in April 2020, was hCoV-19/Brazil/PE-IAM19/2020. The aa change most recently occurred in strain hCoV-19/England/210260926/2021, collected in January 2021. AA change Spike A570D already occurred 18023 times (4.95% of all samples with Spike sequence) in 42 countries. The first strain with this aa change, collected in April 2020, was hCoV-19/Italy/ABR-IZSGC-TE26533/2020. The aa change most recently occurred in strain hCoV-19/Singapore/48/2021, collected in January 2021.