

Genomika i molekularna dijagnostika SARS-CoV-2

Ivan-Christian Kurolt

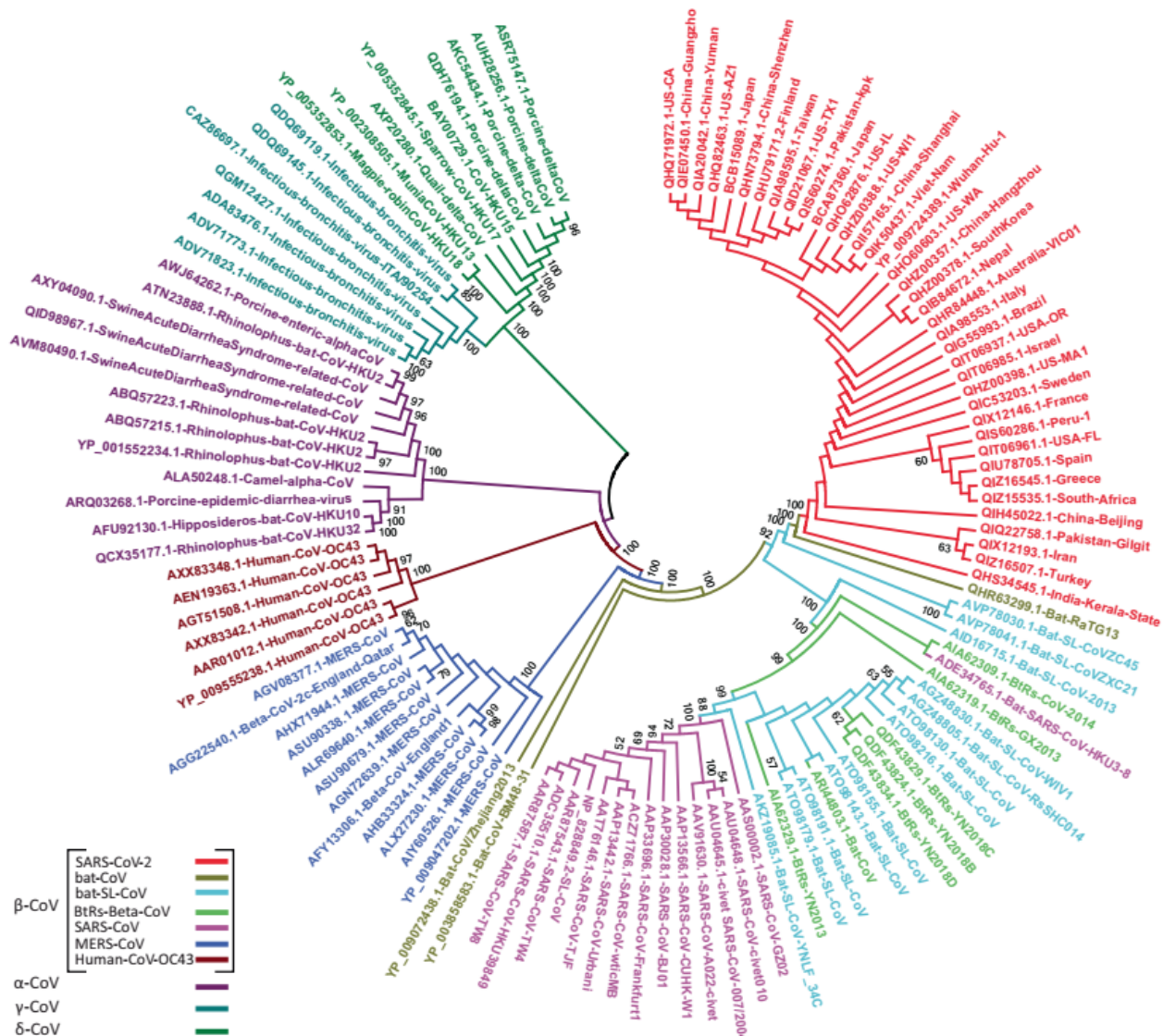
Klinika za infektivne bolesti “Dr. Fran Mihaljević”

Jedinica za znanstvena istraživanja

ikurolt@bfm.hr

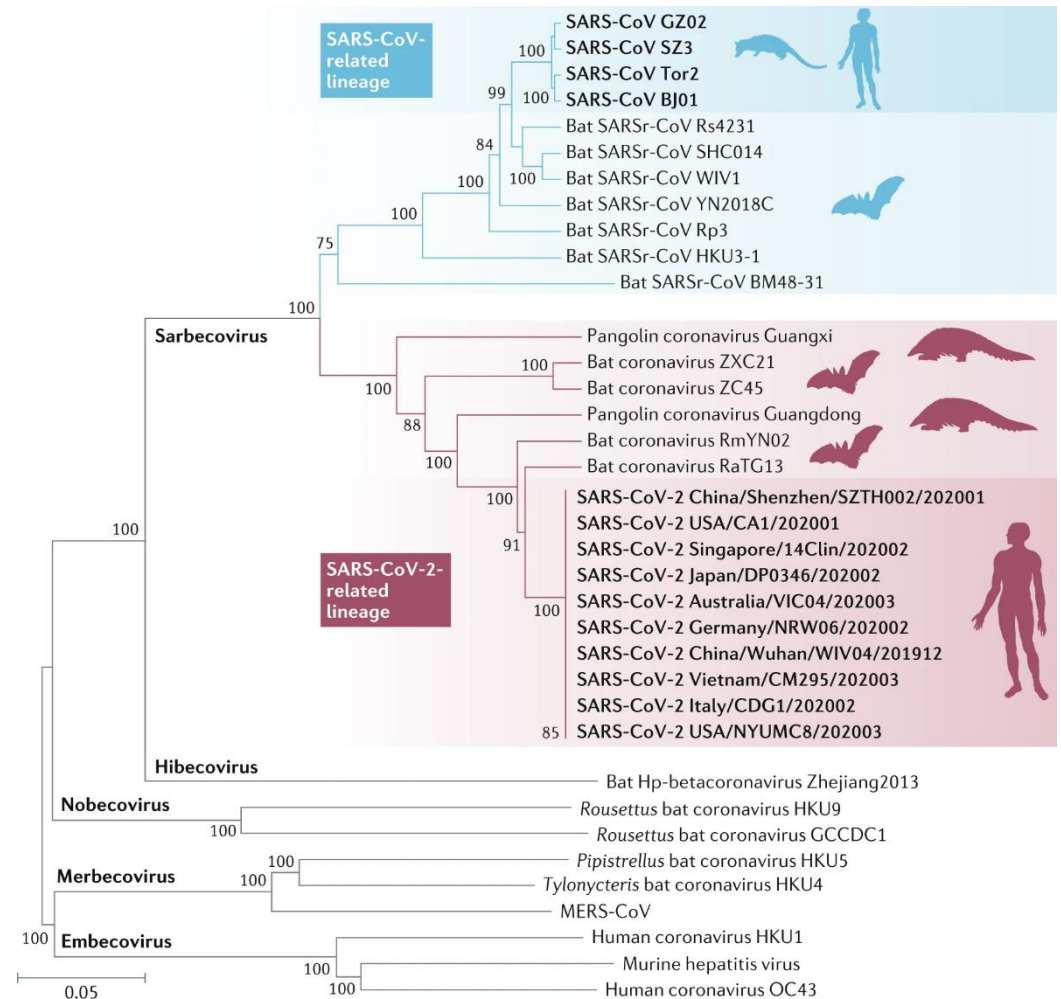


Coronaviridae - Orthocoronavirinae





Coronaviridae - Orthocoronavirinae

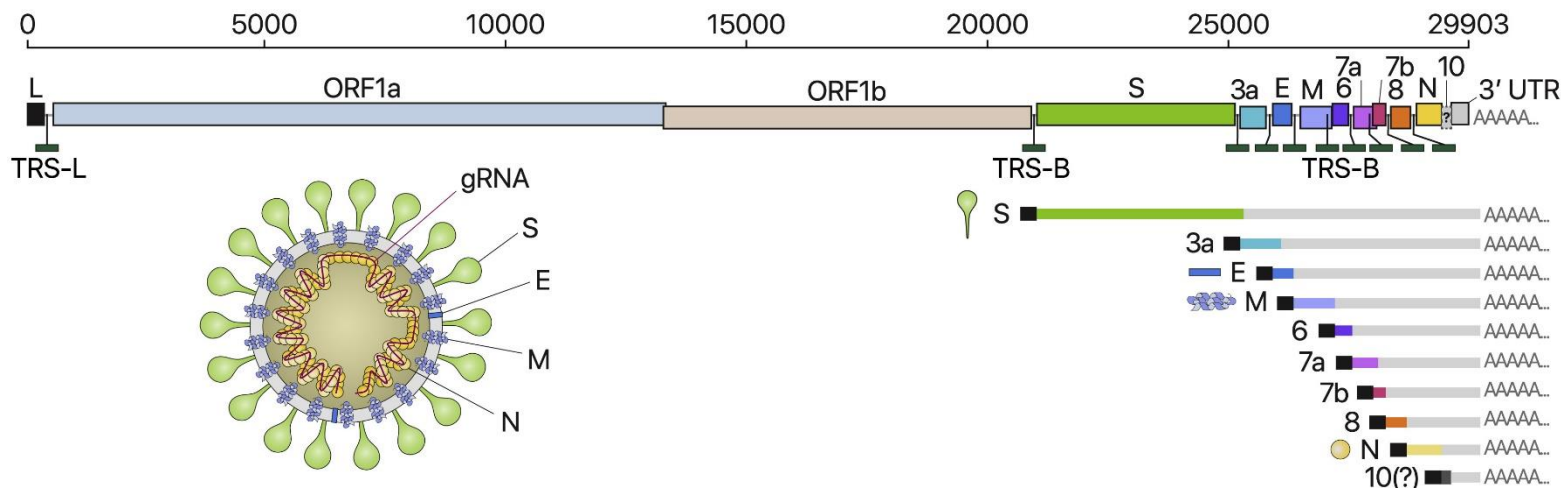


Hu et. al. 2021. *Nature Reviews Microbiology* 19; 141–154



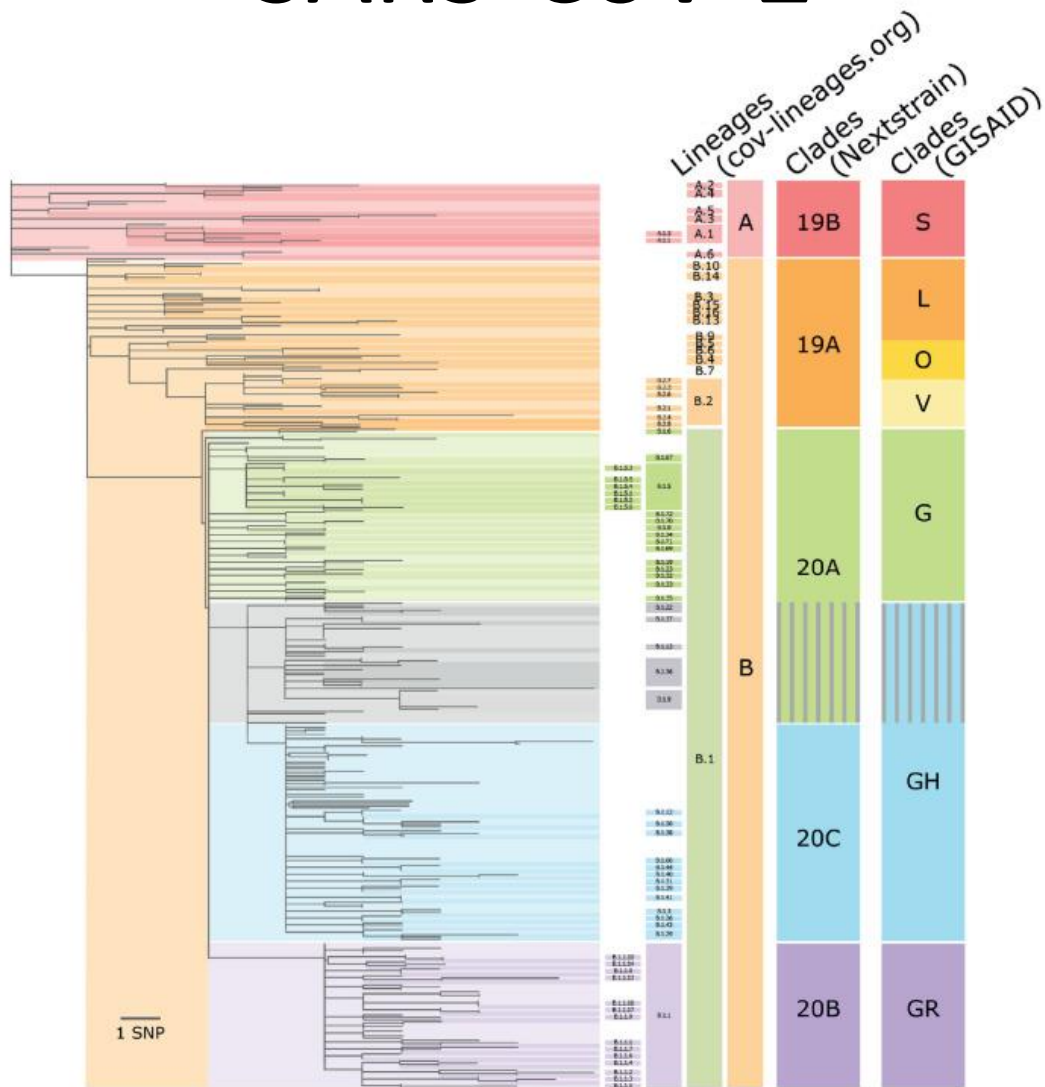
Coronavirus

- + ss RNA genom, 29,9 kB
- 14 ORF, 26 različitih proteina
 - Strukturnih: Spike (S), Envelope (E), Membrane (M), Nucleocapsid (N)
 - 16 nestrukturiranih proteina – prvenstveno potrebno u replikaciji
 - 6 pomoćnih proteina s nepoznatom funkcijom





Filogenetska raznolikost SARS-CoV-2





Implementacija

01.12.2019.: Prvi slučaj COVID-19 u Wuhanu, Kina

21.01.2020.: Odluka o uvođenju testa na COVID-19

27.01.2020.: Isporuka pozitivnih kontrola za COVID-19

29.01.2020.: Isporuka početnica i fluorescentnih proba

30.01.2020.: Zaprimanje prvog uzorka na testiranje COVID-19

WHO PHEIC

14.02.2020.: 1. autohtoni slučaj u Italiji

25.02.2020.: 1. dokazani slučaj u Hrvatskoj





Molekularni testovi za SARS-CoV-2



World Health
Organization

Health Topics ▾

Countries ▾

< Coronavirus disease 2019

< Technical guidance

Laboratory guidance

Early investigations

Patient management

Surveillance and case definitions

Infection prevention and control

Points of entry and mass gatherings

2. Mole

Several assays may

In-house de

Some group
will be willing
recommend

Summary t

Country

China

Germany

Hong Kong

Coronavirus Test Kits Sent to States Are Flawed, C.D.C. Says

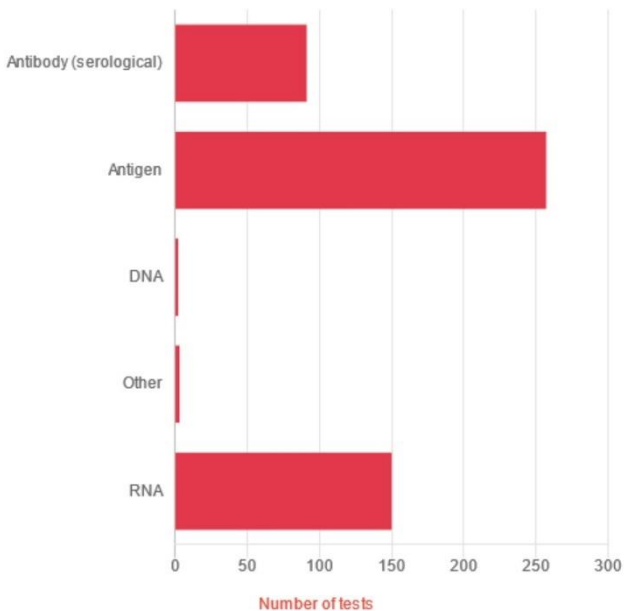
Some tests distributed by the agency deliver “inconclusive” readings. The C.D.C. will need to ship new ingredients, further delaying results.



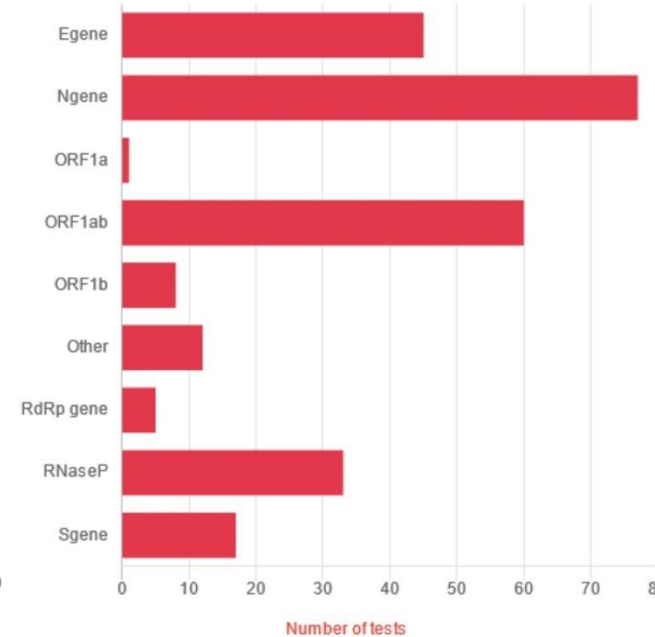


Molekularni testovi za SARS-CoV-2

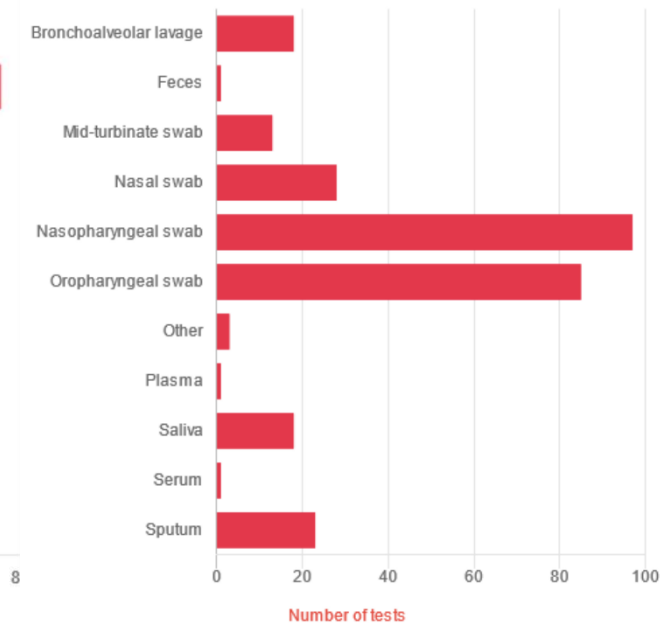
Assay target



Target analyte

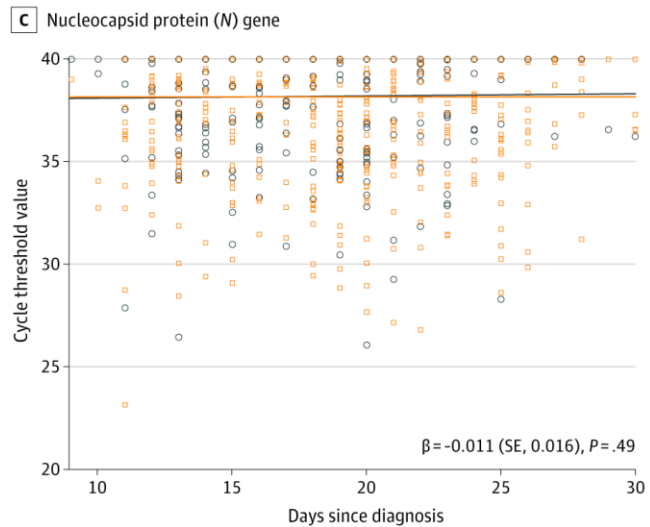
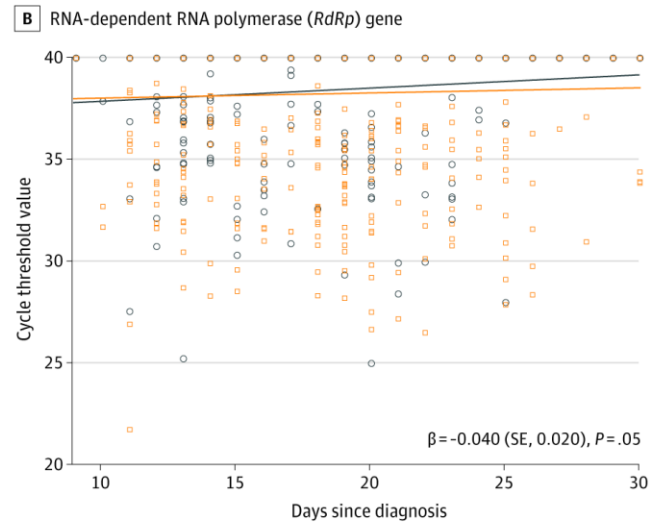
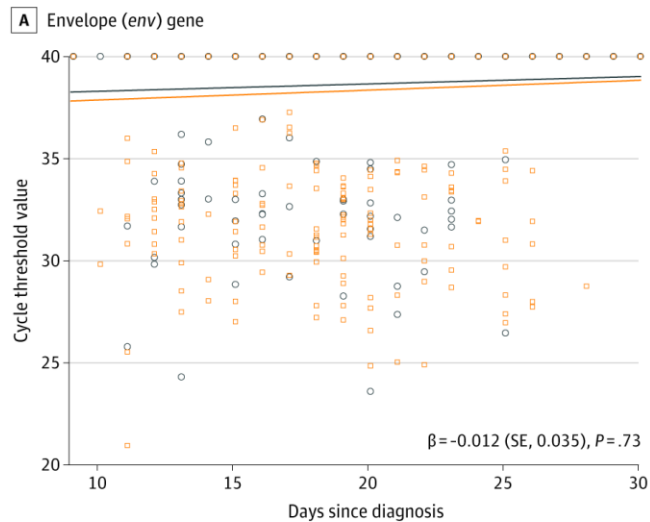


Validated sample types





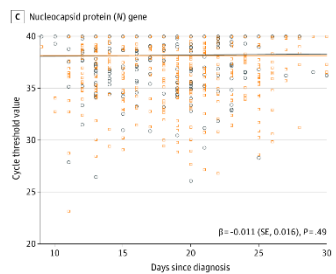
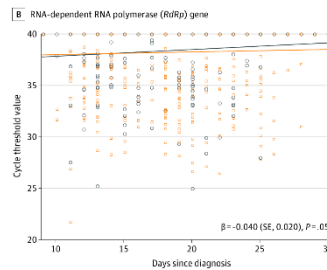
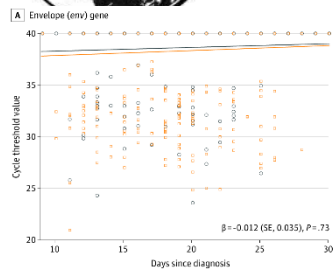
COVID-19 viremija



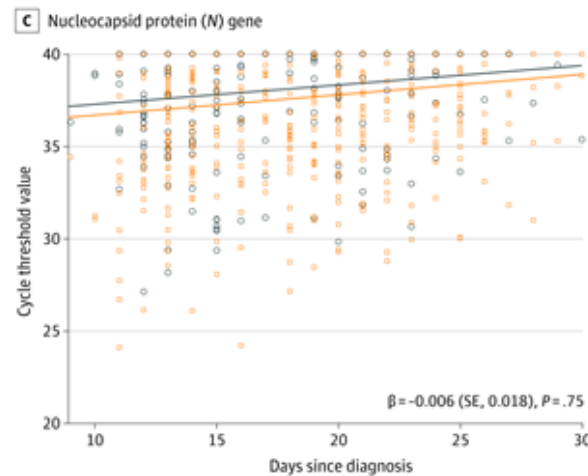
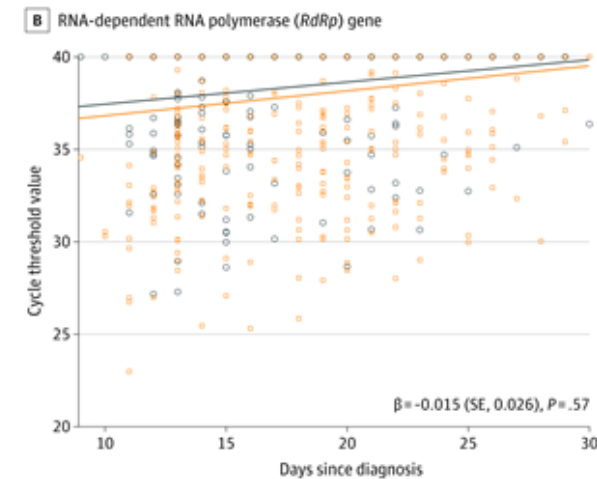
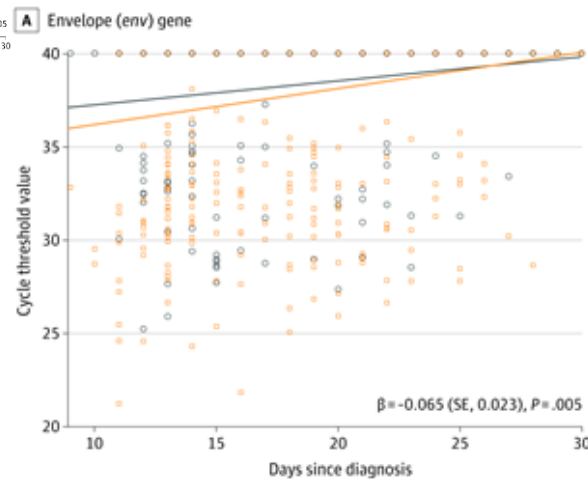
○ Asymptomatic (n=89) — Asymptomatic (n=89)
□ Symptomatic (n=214) — Symptomatic (n=214)
(including presymptomatic) (including presymptomatic)



COVID-19 viremija



○ Asymptomatic (n = 89) — Asymptomatic (n = 89)
 □ Symptomatic (n = 214) — Symptomatic (n = 214)
 (including presymptomatic) (including presymptomatic)

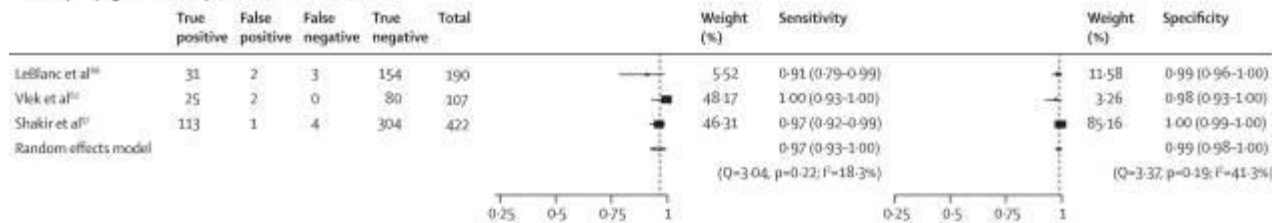


○ Asymptomatic (n = 89) — Asymptomatic (n = 89)
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 (including presymptomatic) (including presymptomatic)

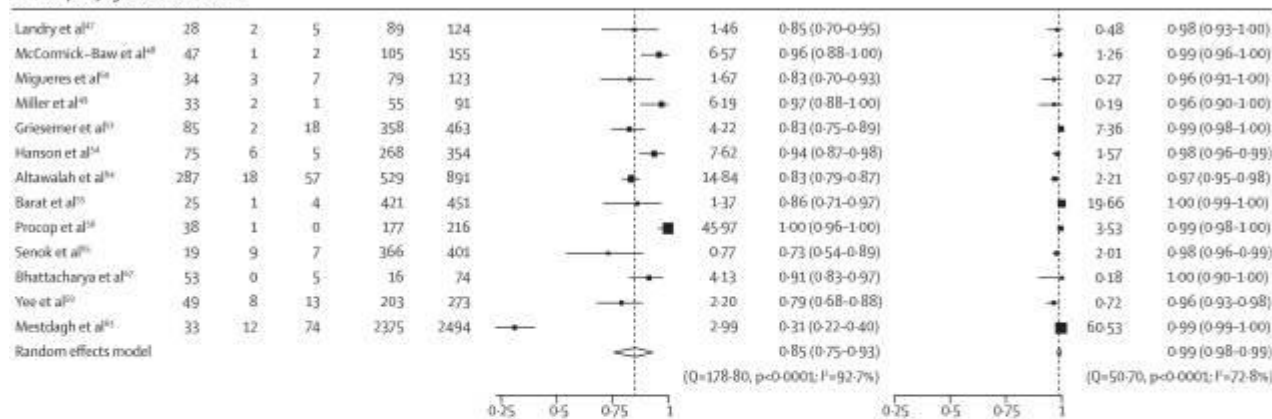


COVID-19 viremija

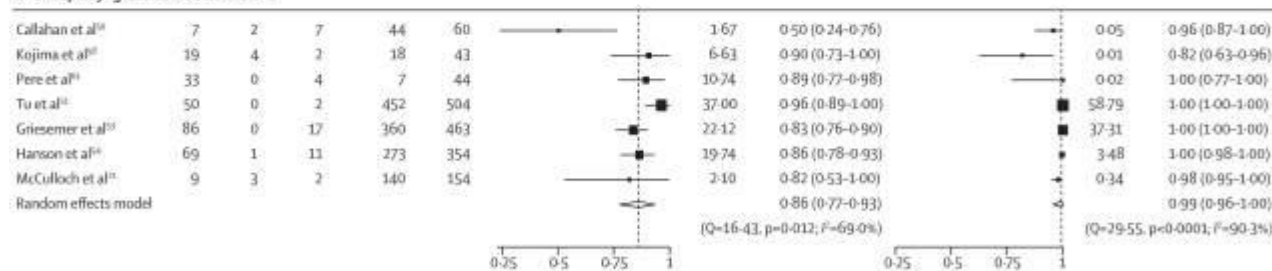
A Nasopharyngeal swab and pooled nasal and throat swab



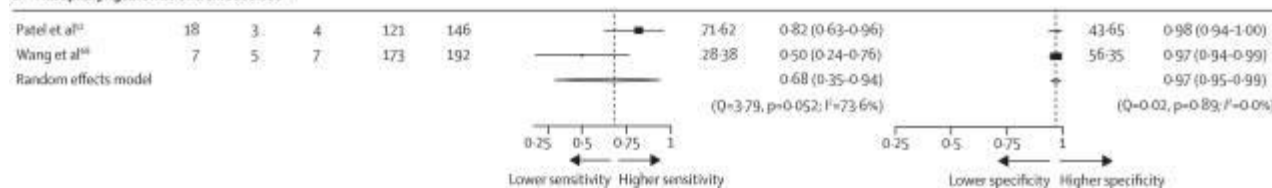
B Nasopharyngeal swab and saliva



C Nasopharyngeal swab and nasal swab



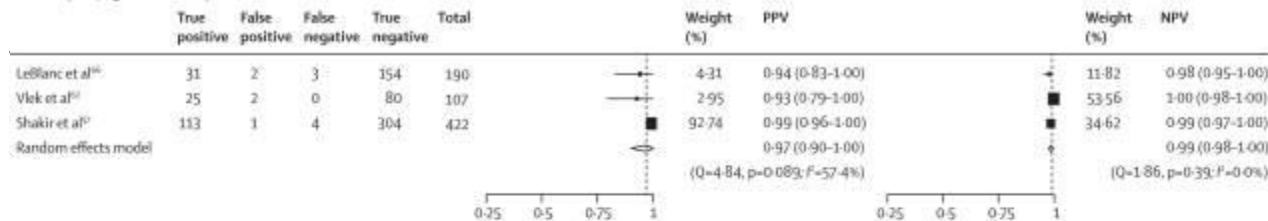
D Nasopharyngeal swab and throat swab



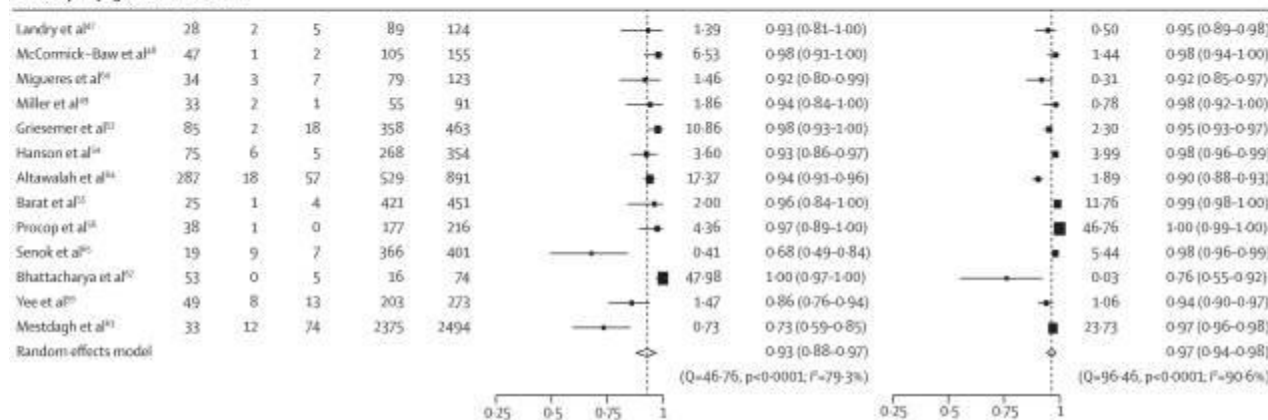


COVID-19 viremija

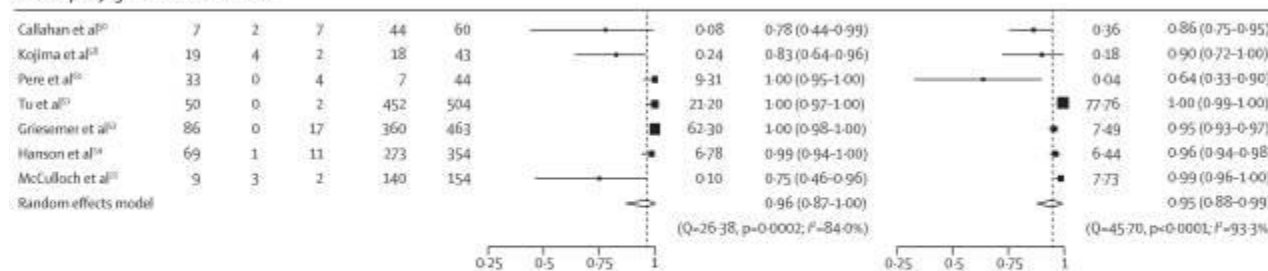
A Nasopharyngeal swab and pooled nasal and throat swab



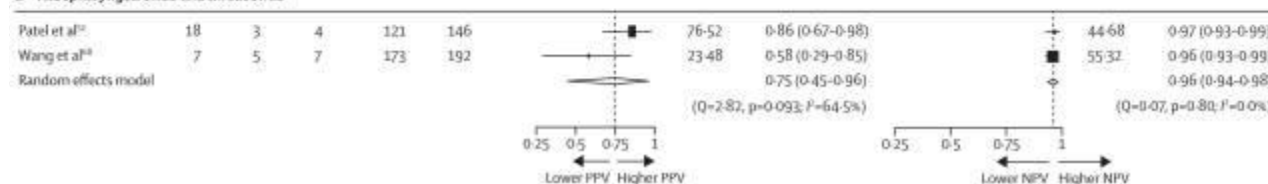
B Nasopharyngeal swab and saliva



C Nasopharyngeal swab and nasal swab

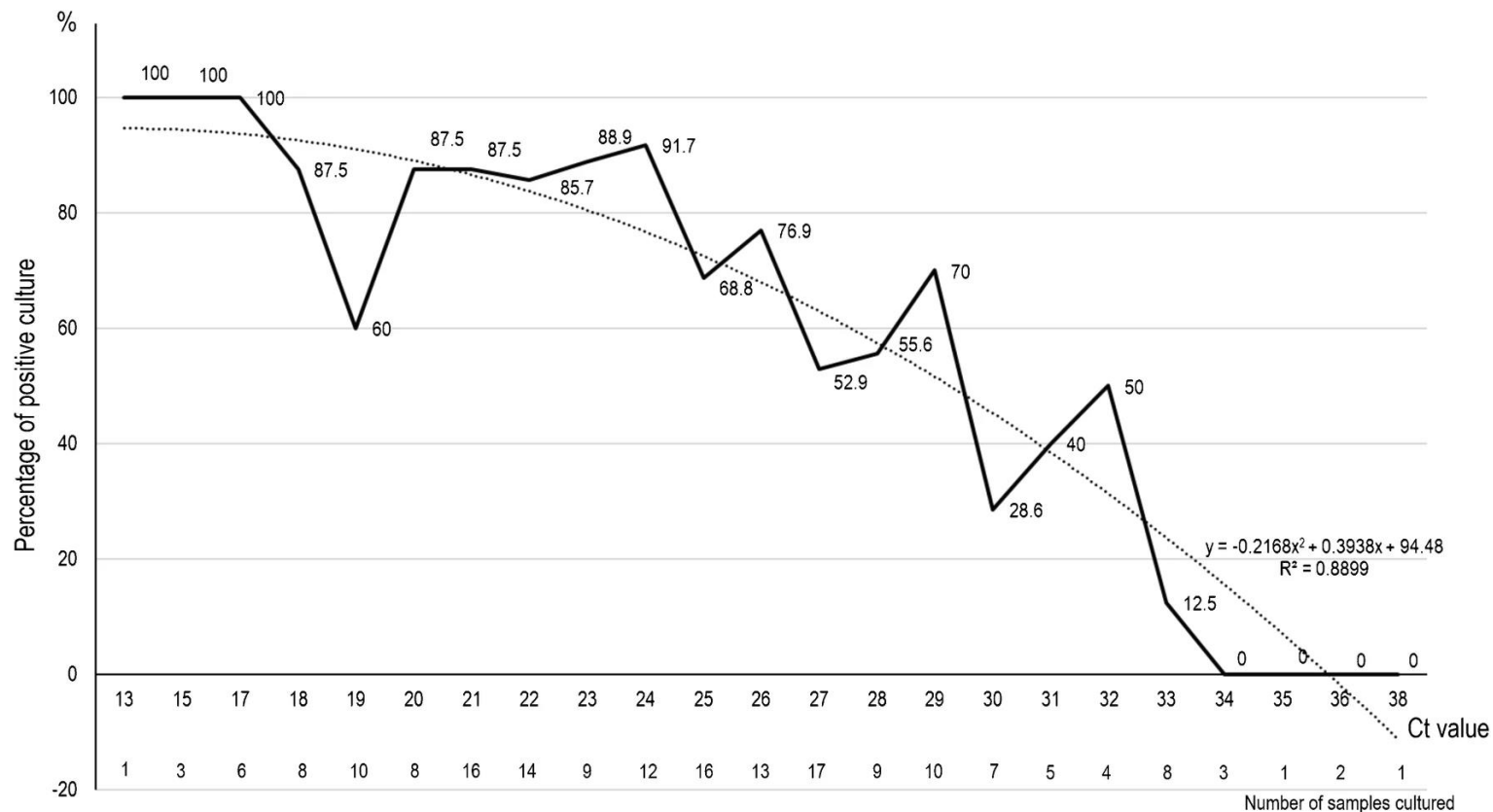


D Nasopharyngeal swab and throat swab



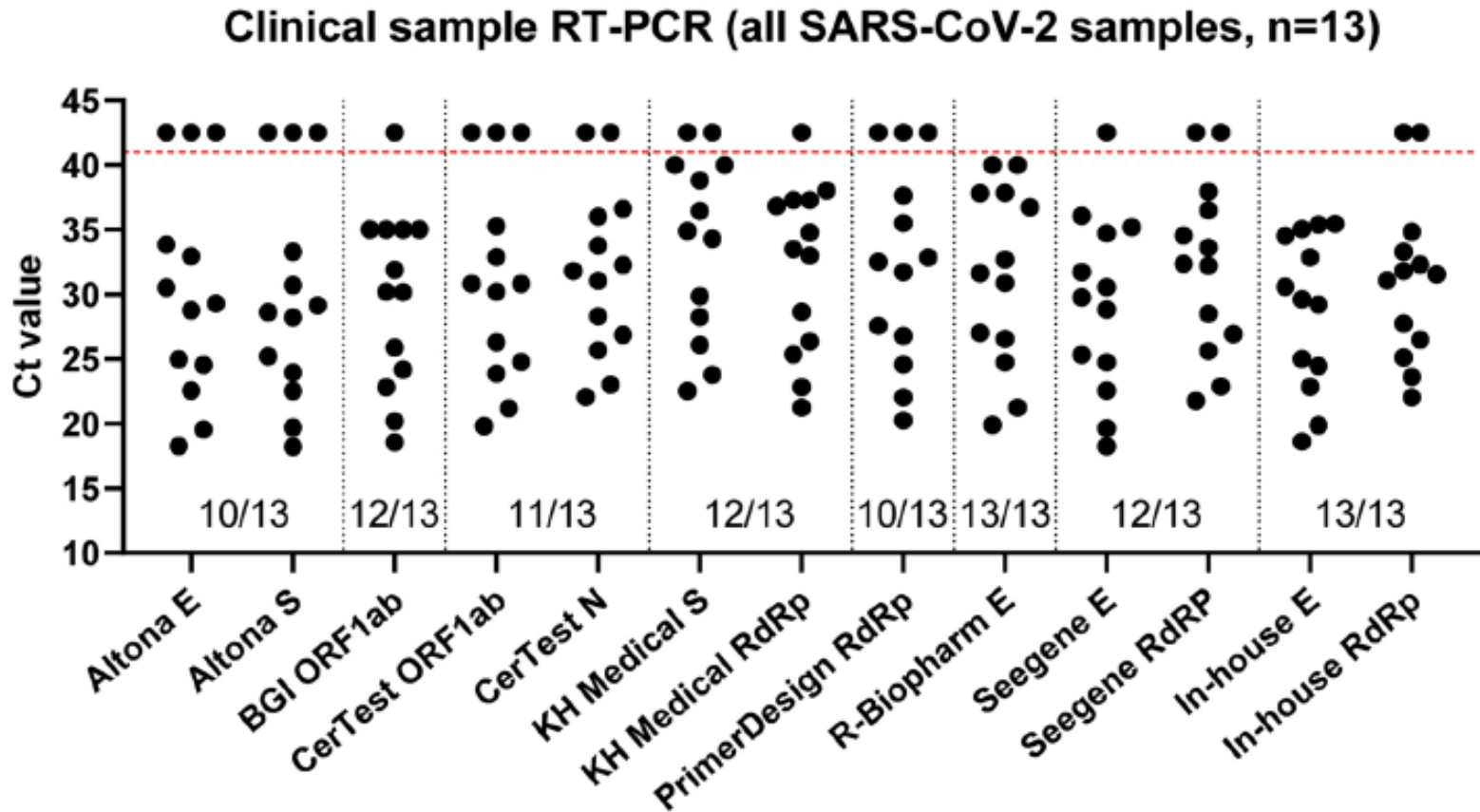


COVID-19 viremija





COVID-19 viremija





COVID-19 viremija

Panel 2	Ref	20	28	Influenza B	RSV	30	34	neg kontrola	21	Influenza A	31	26
Ustanova 1	Rezultat	poz	poz	neg	neg	poz	neg	neg	poz	neg	neg	poz
	gen E	24,5	33,5	-	-	34,5	38,7	-	25,8	-	-	29,2
	gen RDRp	26,3	34,5	-	-	35,3	-	-	27,4	-	-	32
Ustanova 2	Rezultat	poz	neod.	neg	neg	neod.	neg	neg	poz	neg	neod.	poz
	gen E	26	34	-	-	35,5	-	-	28,1	-	35,6	32,8
	gen RdRP	30	-	-	-	-	-	-	33	-	-	38,5
Ustanova 3	Rezultat	poz	poz	neg	neg	poz	neg	neg	poz	neg	neg	poz
	gen E	23	33	-	-	31	38	-	26	-	-	31
	gen RdRp	28	38	-	-	35	-	-	32	-	-	35
Ustanova 4	Rezultat	poz	poz	neg	neg	poz	poz	neg	poz	neg	poz	poz
	gen E	20,54	29,57	-	-	30,40	36,24	-	22,45	-	33,08	27,01
	gen RdRp	22,26	30,97	-	-	32,53	38,60	-	24,44	-	33,90	28,67
Ustanova 5	Rezultat	POZ	POZ	neg	neg	repeat	repeat	neg	POZ	neg	repeat	POZ
	gen: E	25,47	34,56	-	-	36,46	37,24	-	27,48	-	37,19	30,29
	gen: N	24,68	34,00	-	-	36,43	37,26	-	26,72	-	37,08	29,54
	gen: RdRP	26,57	31,48	-	-	-	-	-	28,23	-	-	30,34
Ustanova 6	Rezultat	POZ	GR	NEG	NEG	GR	NEG	NEG	POZ	NEG	GR	POZ
	gen E	22,14	32,21	/	/	34,39	/	/	25,31	/	37,31	28,69
	gen RdRp	23,69	/	/	/	/	/	/	26,76	/	/	29,36



COVID-19 viremija

Barkod Hanks + iClean	Rez Hanks + iClean	Barkod Gongdong Rez Gongdong	Razlika	
0204*577	17,4	0204*649	23,5	6,1
0204*574	16,7	0204*647	24,3	7,6
0204*581	17,6	0204*646	21,7	4,1
0604*258	18	0604*335	23,7	5,7
0604*280	26,2	0604*349	32,9	6,7
0604*448	21,6	0604*611	26,5	4,9
0604*453	18,4	0604*626	23,5	5,1
0604*547	20	0604*729	23,5	3,5
0604*607	16,5	0604*738	25,2	8,7
0604*526	16,2	0604*741	24,4	8,2
0604*541	22,5	0604*742	33,2	10,7
0604*483	25,8	0604*744	28	2,2
0604*607	16,5	0604*738	25,2	8,7
0604*526	16,2	0604*741	24,4	8,2
0604*541	22,5	0604*742	33,2	10,7
0604*483	25,8	0604*745	28	2,2

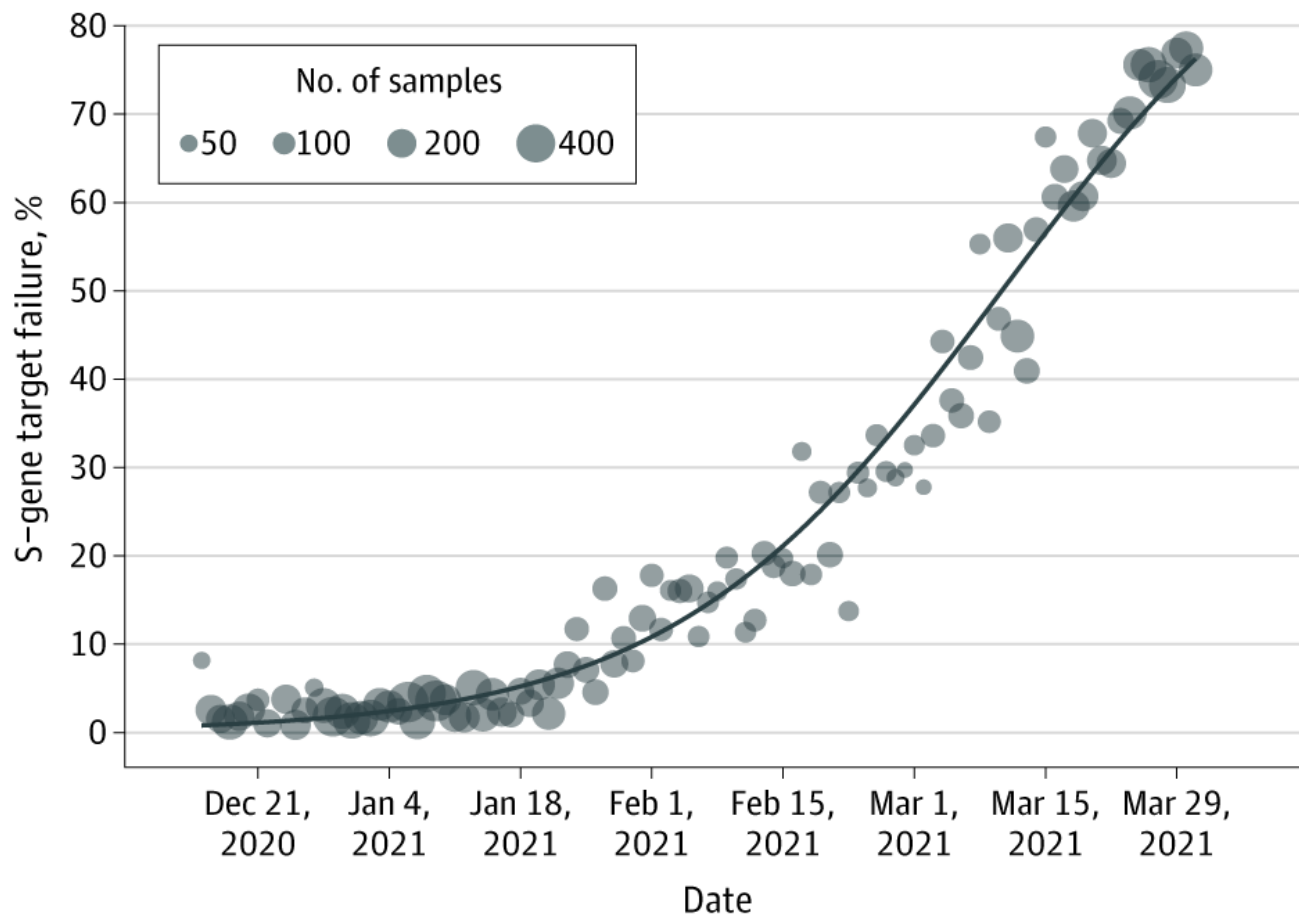


COVID-19 viremija

Barkod Hanks + Gondong štapić	Rez Hanks + Gondong štapić	Barkod Gongdong mediji + iClean štapić	Rez Gongdong mediji + iClean štapić	Razlika
0704*210	22,6	0704*322	27,9	5,3
0704*211	21,4	0704*327	31,7	10,3
0704*241	23,8	0704*318	26,4	2,6
0704*310	19,3	0704*433	26,1	6,8
0704*352	35,1	0704*435	37	1,9
0704*448	29,3	0704*534	29,9	0,6
0704*458	20,5	0704*530	24,9	4,4
0704*510	15,5	0704*550	21,4	5,9
0704*513	20,3	0704*556	29,8	9,5
0704*514	18,1	0704*558	23,4	5,3



COVID-19 viremija



Brown et al. JAMA. Published online April 8, 2021



Antigenski testovi

SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial...

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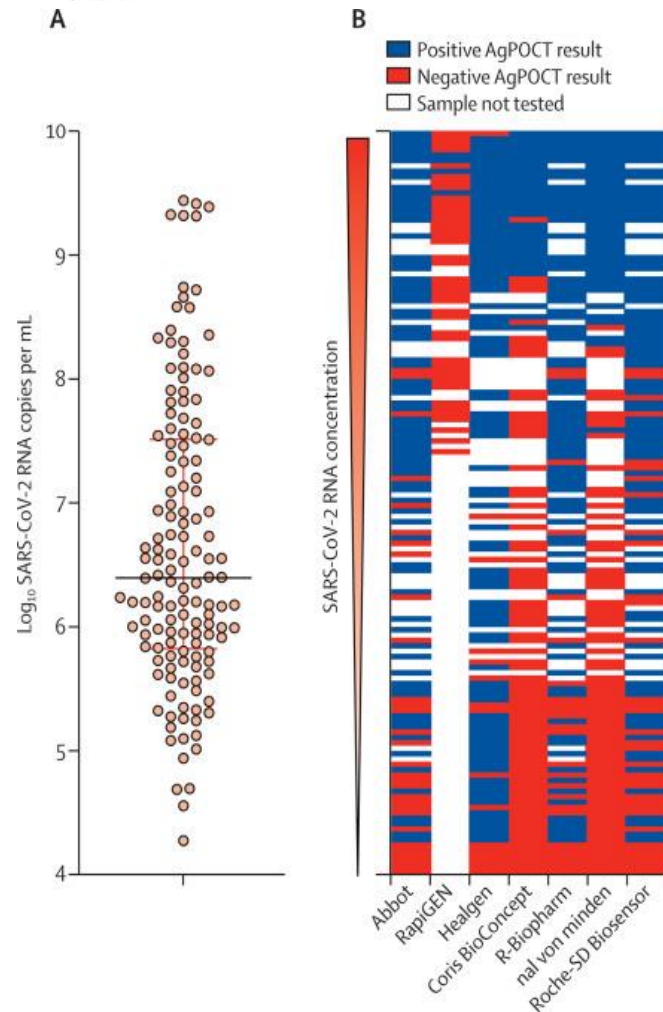


Table 2 Antigen-based detection methods utilized for COVID-19 diagnostics

Ref.	Detection method	Antigen	Sample type	Num. of samples	Days since symptom onset (days)	Sensitivity (%) LOD	Specificity (%)
[106]	Fluorescence immunochromatographic assay	Nucleocapsid protein	NPS and urine	239	3	68	100
[115]	Fluorescence immunochromatographic assay	Nucleocapsid protein	Saliva	103	9	11.7	N/A
[116]	Chemiluminescence enzyme immunoassay	Nucleocapsid protein	NPS	313	N/A	55.2	99.6
[117]	Fluorescence immunochromatographic assay	Nucleocapsid protein (Genscript Cat #Z03488 & Genemedi GMP-V-2019nCoV-N002)	Non-clinical samples (in PBS buffer)	N/A	N/A	Genemedi – 0.65 ng/mL, Genscript – 3.03 ng/mL	N/A
[118]	Fluorescence immunochromatographic assay	SARS-CoV-2 antigen	NPS	19	N/A	N/A (low)	N/A
[114]	Fluorescence immunochromatographic assay	SARS-CoV-2 antigen	NPS and OPS	127	<7 for 93.7% of samples	93.9	100
[119]	GICA	Nucleoprotein	NPS	138	N/A	50	100
[120]	GICA	Nucleoprotein	NPS	148	Median: 4, mean: 6.6, range: 0–34	30.2	100
[121]	GICA	Nucleoprotein	NPS	328	N/A	57.6	99.5

Jayamohan et al. Anal Bioanal Chem. 2021;413(1):49-71

Corman et al. Lancet Microbe 2021 Apr 7



Serologija

Table 3 Antibody-based tests utilized for COVID-19 diagnostics

Ref.	Detection method	Antibody	Sample type	Num. of samples	Seroconversion (days)	Sensitivity	Specificity
[129]	GICA	IgG and IgM	Serum/whole blood	134	7	96.8 ¹	N/A
[130]	GICA	IgG and IgM	Serum/whole blood	525	N/A	88.66 ²	90.63 ²
[131]	GICA	IgG and IgM	Serum	814	5	86.89 ²	99.39 ²
[132]	GICA	IgG and IgM	Serum	179	8	95.10 ²	91 ²
[125]	CLIA	IgG and IgM	Serum	285	13	100 ¹	N/A
[123]	CLIA	IgG and IgM	Serum	159	14	91.14 ²	80 ²
[124]	CLIA	IgG and IgM for nucleocapsid protein	Serum	222	4	81.5 ²	96.6 ²
[133]	ELISA	IgG and IgM	Serum	238	11	81.3 ²	N/A
[134]	ELISA	IgG and IgM for nucleocapsid and spike protein	Serum	214	10	82.2	N/A
[135]	ELISA	IgG and IgM	Serum	15	5	N/A	N/A
[136]	ELISA	IgA, IgM, and IgG	Serum	208	5	85.4	N/A
[126]	ELISA	IgG, IgA for spike protein	Serum	61	N/A	N/A	N/A

¹ Highest sensitivity among samples tested

² Sensitivity reported as a mean of all samples tested

N/A. data not reported or not relevant in the context of the referenced publication

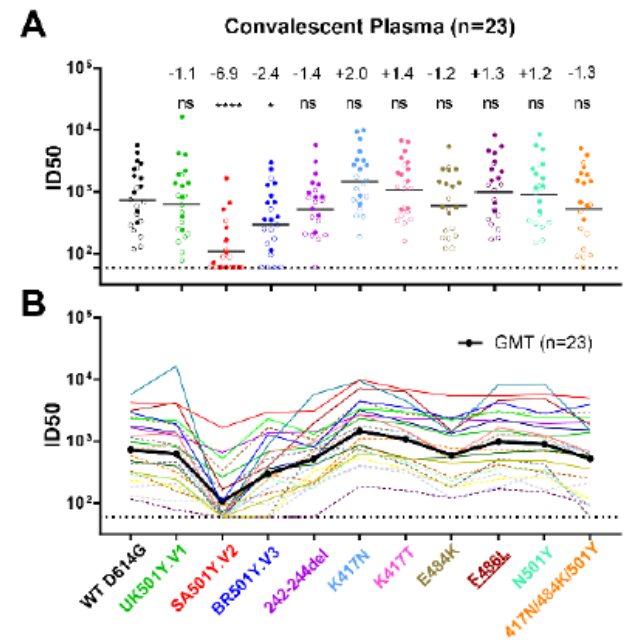
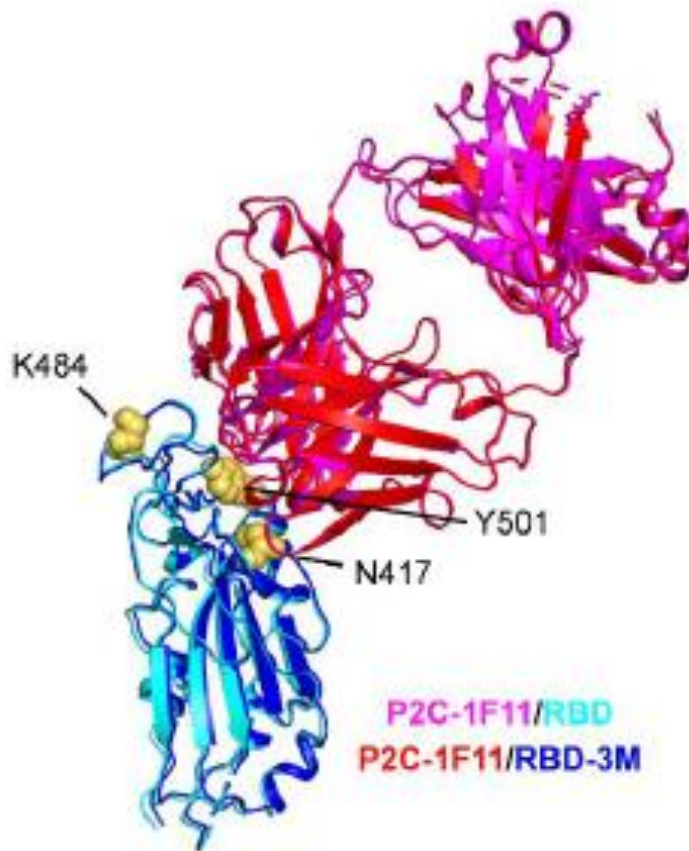


Varijante

Pango lineage	Nextstrain	Mutacije Spike	Lokacija	Svojstva	Varijanta
B.1.617		E484Q, L452R, P681R	India	increased transmission	Vul
B.1.526	20C/S:484K	(L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*)	NY	Reduced neutralization	Vol
B.1.526.1	20C	D80G, Δ144, F157S, L452R, D614G, (T791I*), (T859N*), D950H	NY	Reduced neutralization	Vol
B.1.525	20A/S:484K	A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L	UK/Nigeria	Potential reduction in neutralization	Vol
P.2	20J	E484K, (F565L*), D614G, V1176F	Brazil	Reduced neutralization by post-vaccination sera	Vol
B.1.1.7	20I	Δ69/70, Δ144, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)	UK	50% increased transmission Likely increased severity	VoC
P.1	20J/501Y.V3	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027	Japan/Brazil	Reduced neutralization	VoC
B.1.351	20H/501.V2	D80A, D215G, Δ241/242/243, K417N, E484K, N501Y, D614G, A701V	SA	50% increased transmission Reduced neutralization	VoC
B.1.427	20C/S:452R	L452R, D614G	US-Cal	20% increased transmissibility Reduced neutralization	VoC
B.1.429	20C/S:452R	S13I, W152C, L452R, D614G	US-Cal	20% increased transmissibility Reduced neutralization	VoC



Varijante



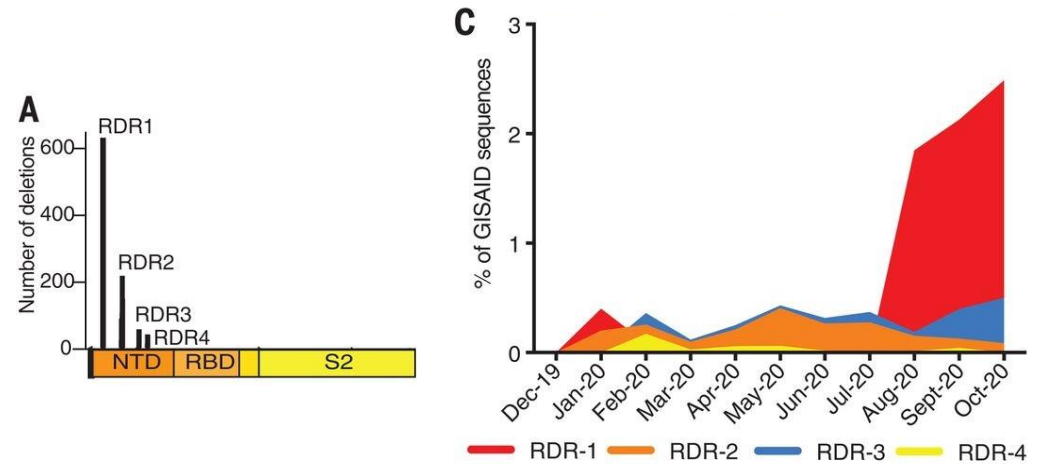
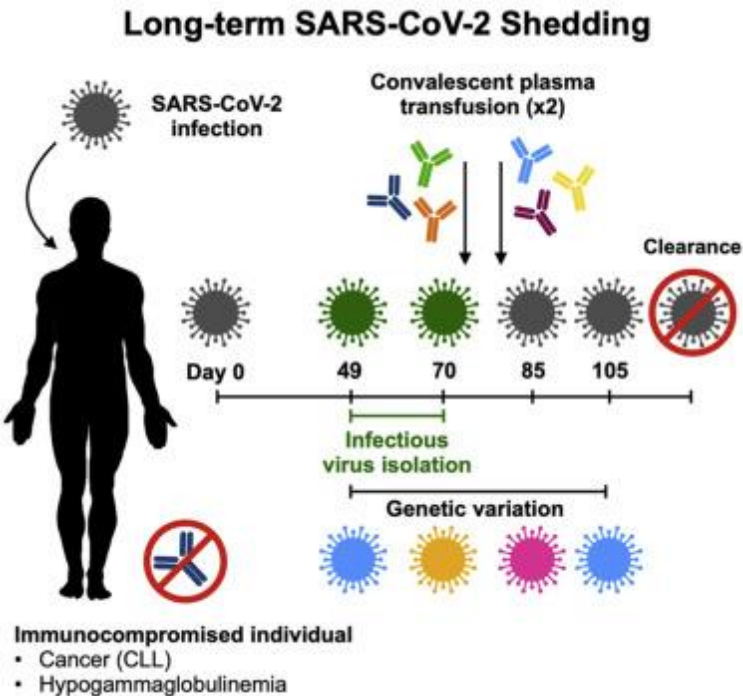
Fold changes in ID50	WT	Variants				NTD		RBD			
C-Plasma1	+1.0	-1.5	-27.6	-2.3	-3.6	+1.5	+1.2	-1.2	+1.5	-1.0	+1.4
C-Plasma2	+1.0	-1.1	-2.6	-1.4	-1.4	+2.3	+1.6	+1.3	+1.3	+1.3	+1.2
C-Plasma3	+1.0	-1.2	-3.7	-1.4	-1.2	+2.4	+1.9	+1.3	+1.6	+1.2	+1.4
C-Plasma4	+1.0	-1.3	-2.5	-1.2	-1.2	+1.6	+1.4	+1.4	+1.3	+1.2	+1.2
C-Plasma5	+1.0	-1.8	-6.7	-2.0	-2.7	+1.9	+1.9	+1.3	+1.3	+1.2	-1.0
C-Plasma6	+1.0	-1.3	-7.2	-2.2	-1.6	+1.5	+1.2	-2.0	+1.1	+1.1	-1.9
C-Plasma7	+1.0	-1.3	-7.2	-1.5	-2.3	+1.8	+1.1	+1.0	+1.2	+1.2	+1.2
C-Plasma8	+1.0	-1.3	BDL	-5.0	-3.4	+1.8	+1.1	-1.3	+1.3	-1.1	-1.2
C-Plasma9	+1.0	-1.5	BDL	BDL	BDL	+1.6	+1.4	+1.0	+1.4	+1.3	-1.2
C-Plasma10	+1.0	+1.3	-18.7	-8.0	-1.6	+2.3	+2.0	-2.1	+1.5	+1.6	-1.7
C-Plasma11	+1.0	-1.2	-6.7	-1.4	-1.1	+1.8	+1.1	+1.1	+1.4	+1.5	+1.3
C-Plasma12	+1.0	+1.1	BDL	-3.1	-1.1	+2.0	+1.5	-1.4	+1.7	+1.5	-1.6
C-Plasma13	+1.0	-1.1	-4.4	-1.0	-1.8	+1.4	+1.3	-1.0	+1.3	+1.0	+1.1
C-Plasma14	+1.0	-1.1	BDL	-3.9	-1.8	+2.0	+1.8	-1.2	+1.5	+1.4	-1.0
C-Plasma15	+1.0	+1.1	BDL	BDL	+1.1	+2.2	+1.8	-1.5	-1.0	+1.7	-2.1
C-Plasma16	+1.0	+1.1	BDL	BDL	+1.8	+1.7	-1.0	-2.5	+1.2	-1.0	BDL
C-Plasma17	+1.0	-1.3	BDL	BDL	+1.4	+2.3	+1.5	-1.3	+1.0	+1.1	-2.0
C-Plasma18	+1.0	-2.1	-3.7	-2.3	-1.6	+2.5	+1.6	-1.3	+1.3	-1.0	-1.3
C-Plasma19	+1.0	-1.2	-1.1	-1.4	+1.5	+3.0	+2.4	+1.5	+2.3	+1.3	+1.6
C-Plasma20	+1.0	-1.3	BDL	-2.8	-1.4	+2.0	+1.7	+1.4	+1.5	+1.5	+1.1
C-Plasma P#2	+1.0	+2.9	BDL	-6.5	+1.0	+1.6	-1.3	-4.1	+1.4	+1.5	-3.8
C-Plasma P#5	+1.0	+1.1	BDL	-1.9	-1.3	+2.2	+1.2	-2.2	+1.4	+1.0	-1.8
C-Plasma P#22	+1.0	-1.1	BDL	-1.1	-1.7	+2.4	+1.6	-1.2	+1.3	+1.2	-3.1
C-Plasma Standard	+1.0	BDL	BDL	+4.3	BDL	+1.5	+1.1	+1.3	+2.0	+1.1	+1.0

WT D614G
UK501Y.V1
SA501Y.V2
BR501Y.V3
242-244del
K417N
K417T
E484K
E486L
N501Y
417N/484K/501Y

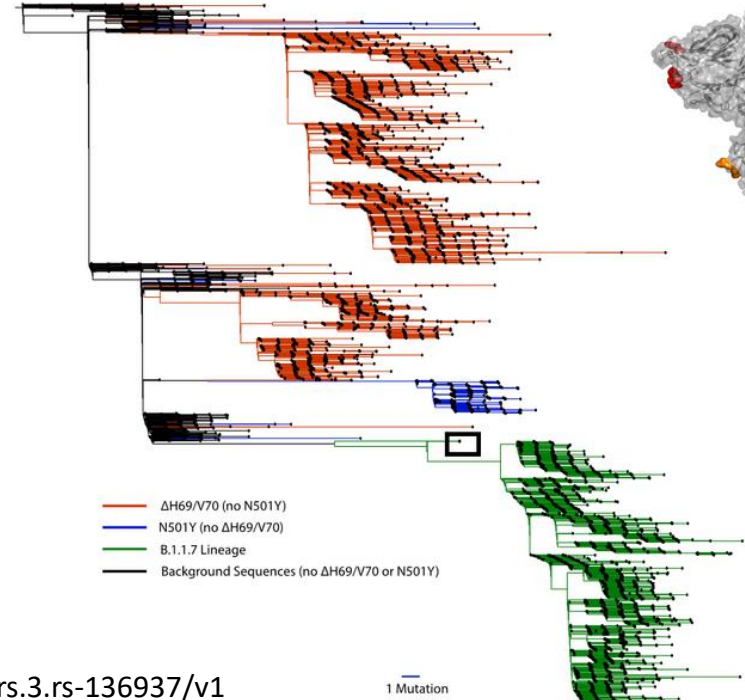
Wang et al. 2021. SARS-CoV-2 variants resist antibody neutralization and broaden host ACE2 usage



Varijante



McCarthy et al. Science. 2021 Mar 12;371(6534):1139-1142

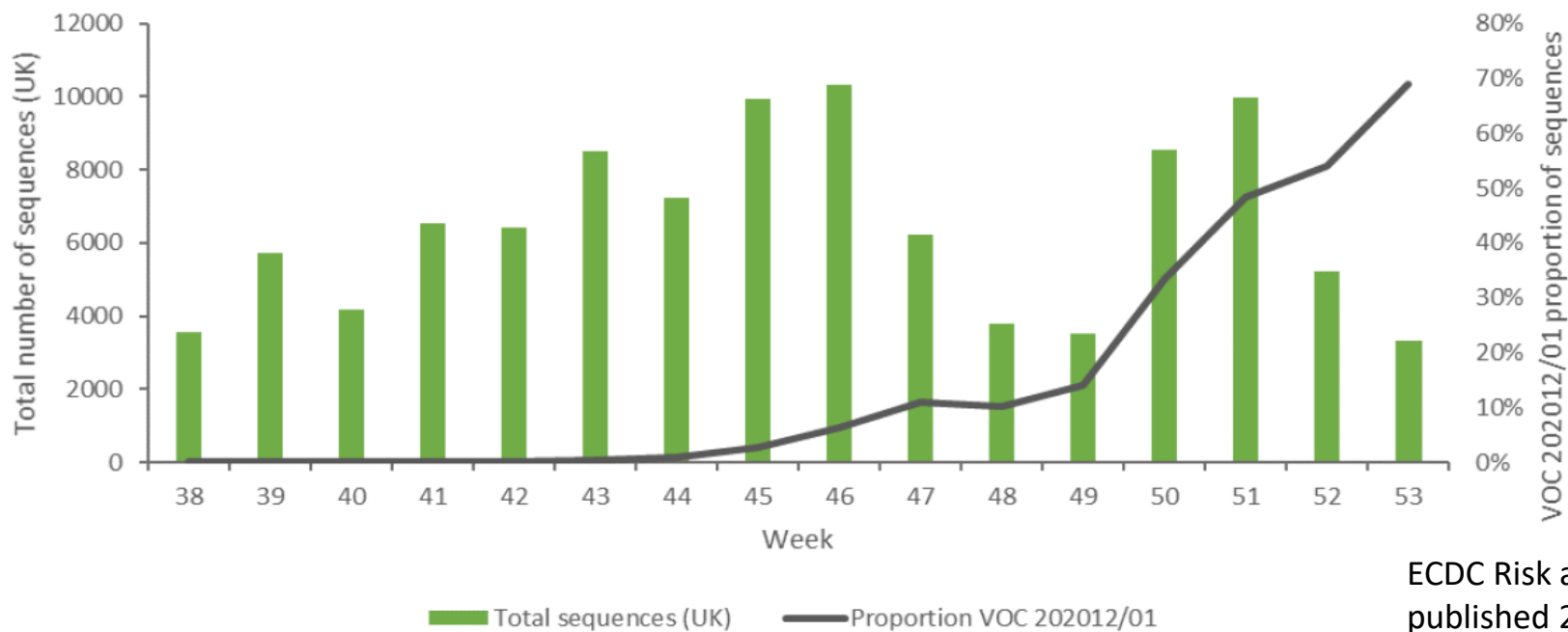
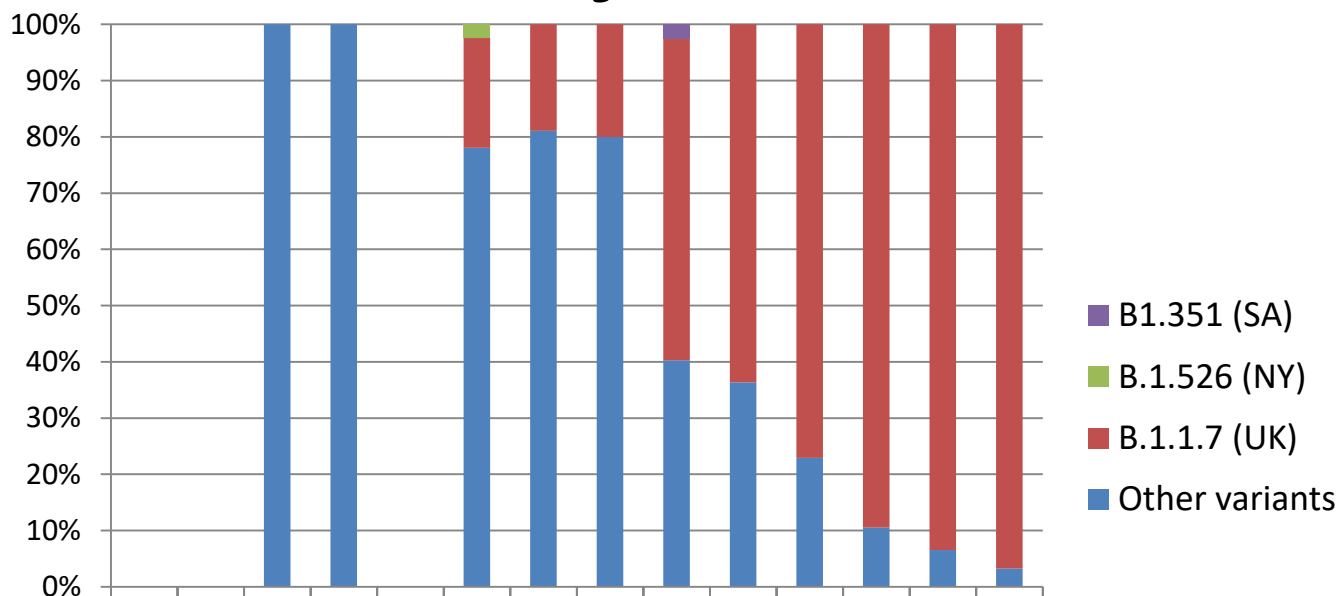


Gupta et al. 10.21203/rs.3.rs-136937/v1

Avanzato et al. Cell. 2020 Dec 23;183(7):1901-1912.e9



Varijante





Hvala na pažnji!

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