Genomika i molekularna dijagnostika SARS-CoV-2

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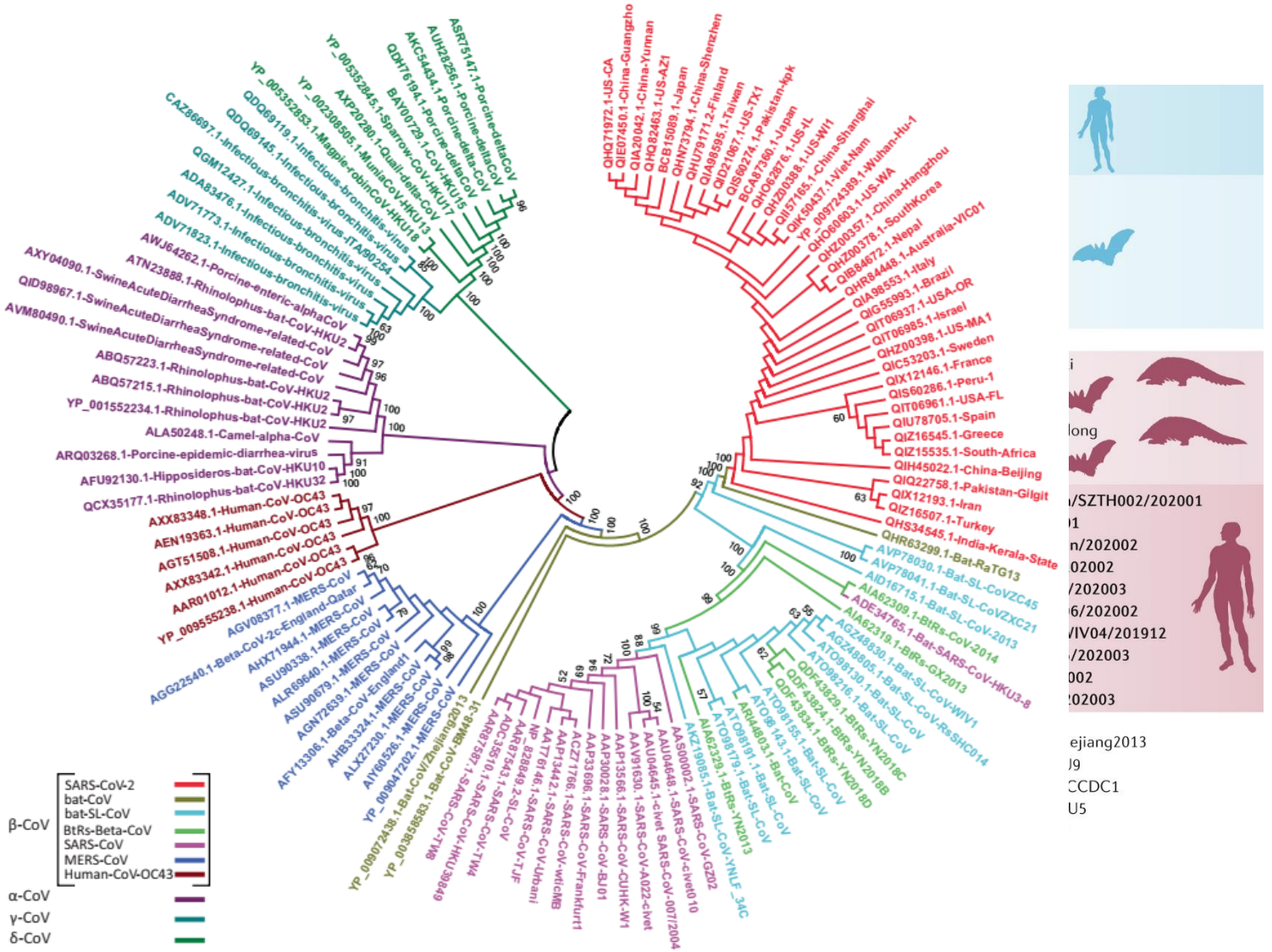
Coronaviridae - Orthocoronavirinae

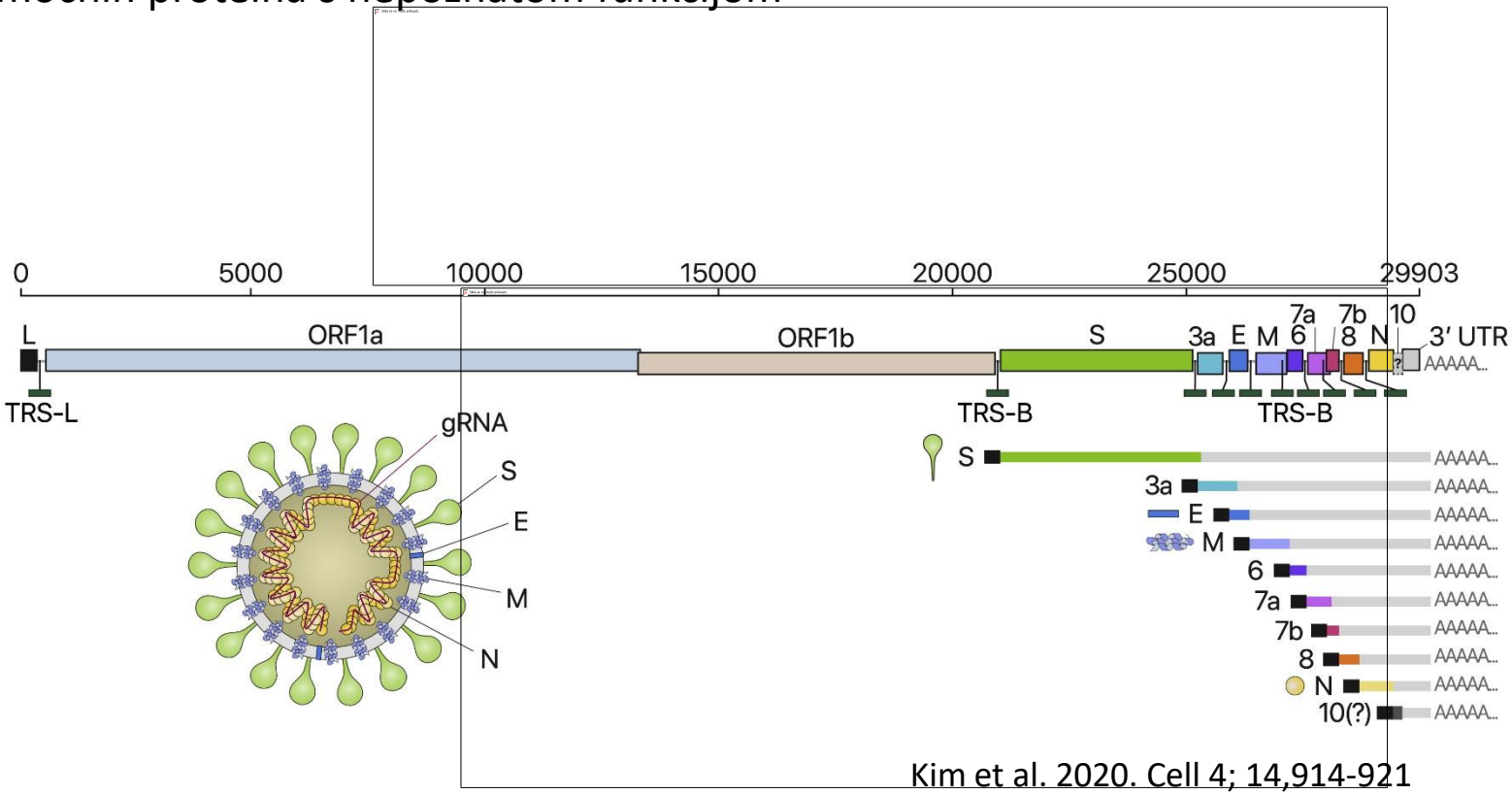


Hussain et al. Genes Immun. 2020 Dec;21(6-8):409-419

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

Coronavirus

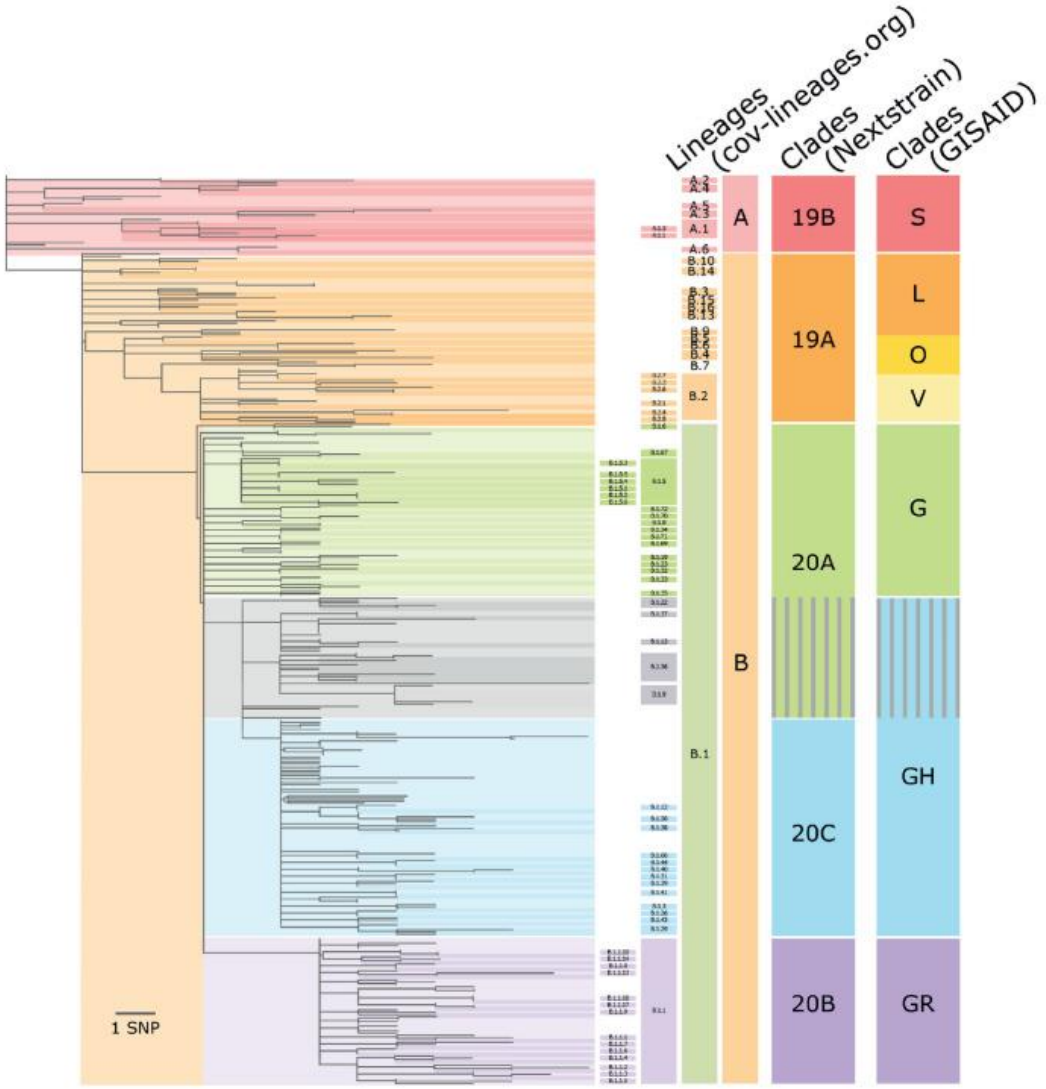
- + ss RNA genom, 29,9 kB
- 14 ORF, 26 različitih proteina
  - Strukturnih: Spike (S), Envelope (E), Membrane (M), Nucleocapsid (N)
  - 16 nestrukturih proteina – prvenstveno potrebno u replikaciji
  - 6 pomočnih proteina s nepoznatom funkcijom



Kim et al. 2020. Cell 4; 14,914-921

Finkel et al. 2021. Nature 589, 125-30

Filogenetska raznolikost SARS-CoV-2



Alm et al. Euro Surveill. 2020 Aug 13; 25(32): 2001410.

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

EVAg
European Virus Archive Global
European Virus Archive - GLOBAL

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

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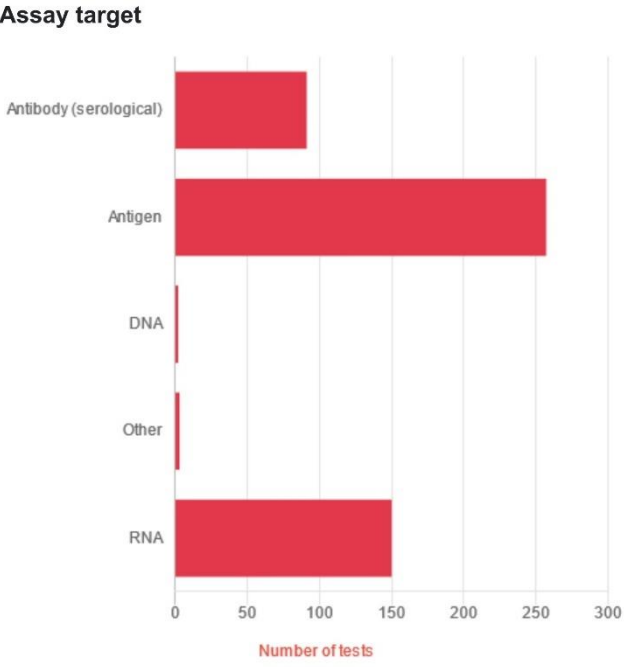
Summary t

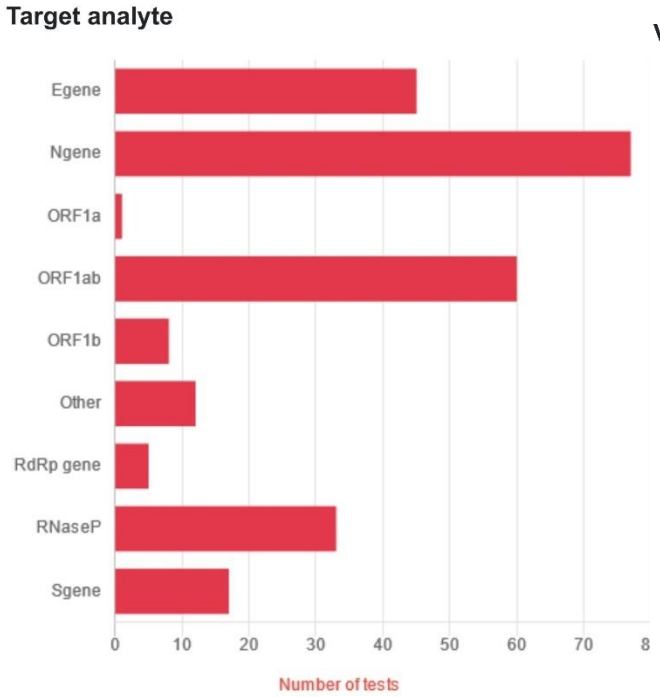
Country
China
Germany
Hong Kong

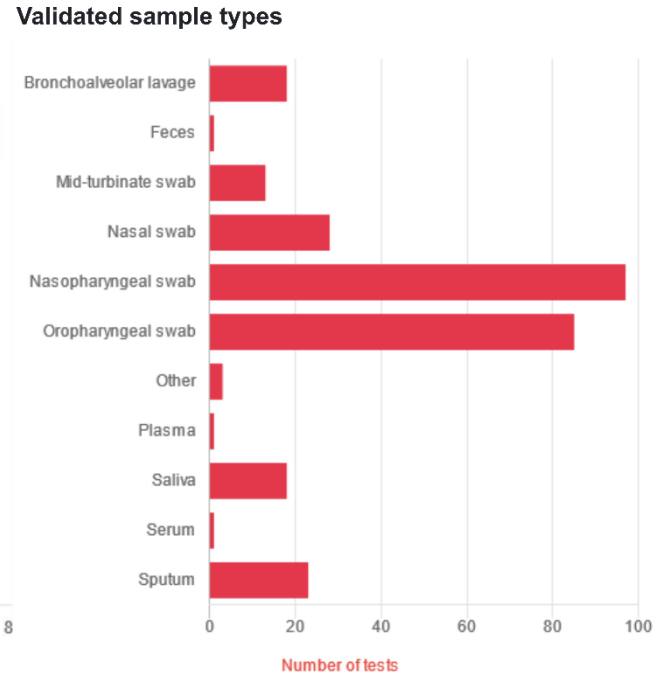
Coronavirus Test Kits Sent to States Are Flawed, C.D.C. Says

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

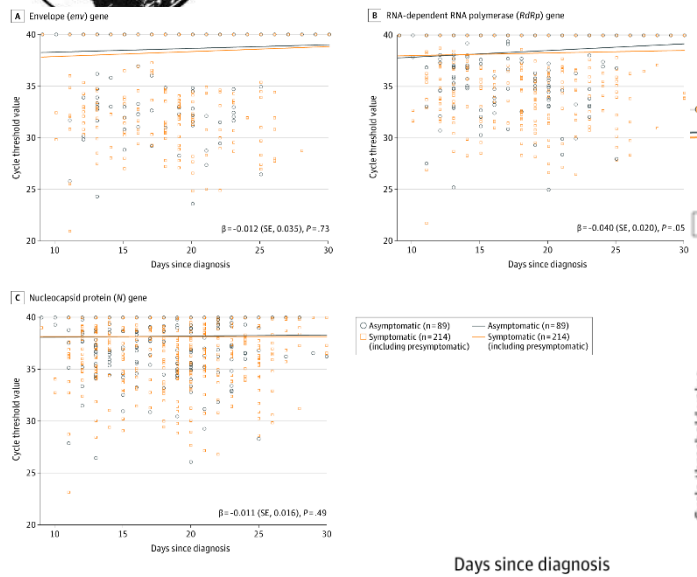


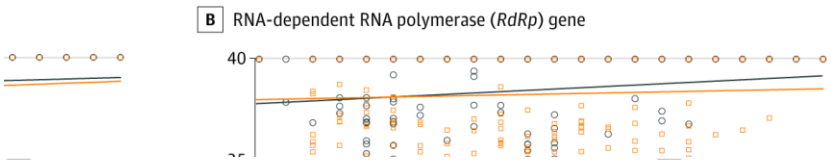


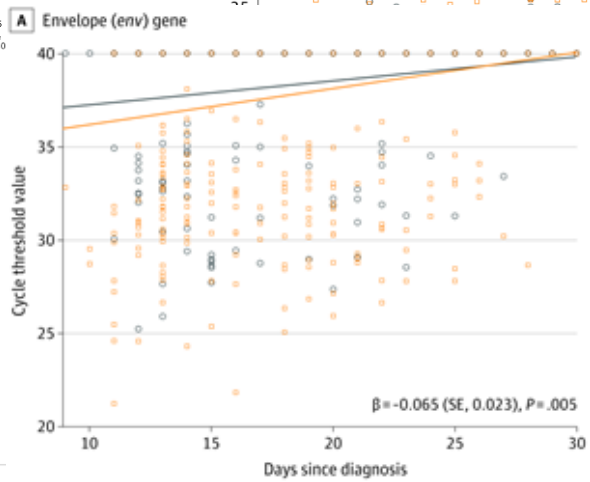


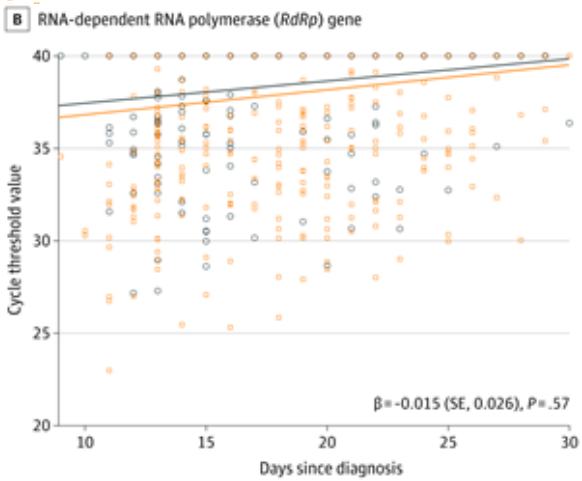
www.finddx.org

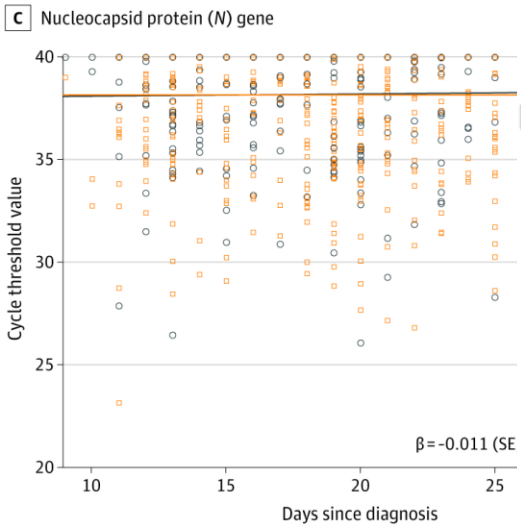
SARS-CoV-2 virusno opterećenje

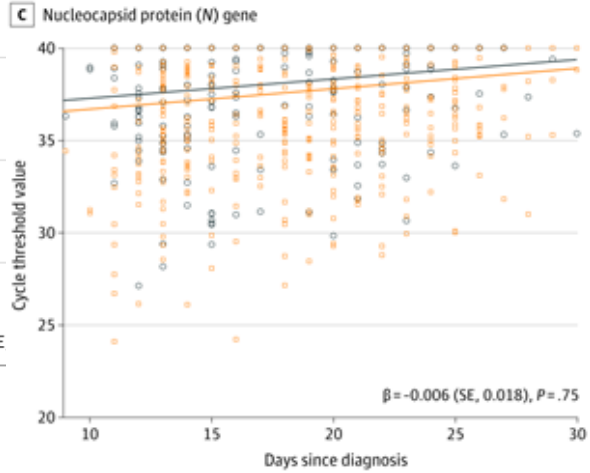


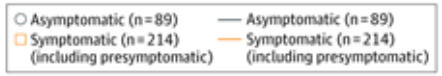






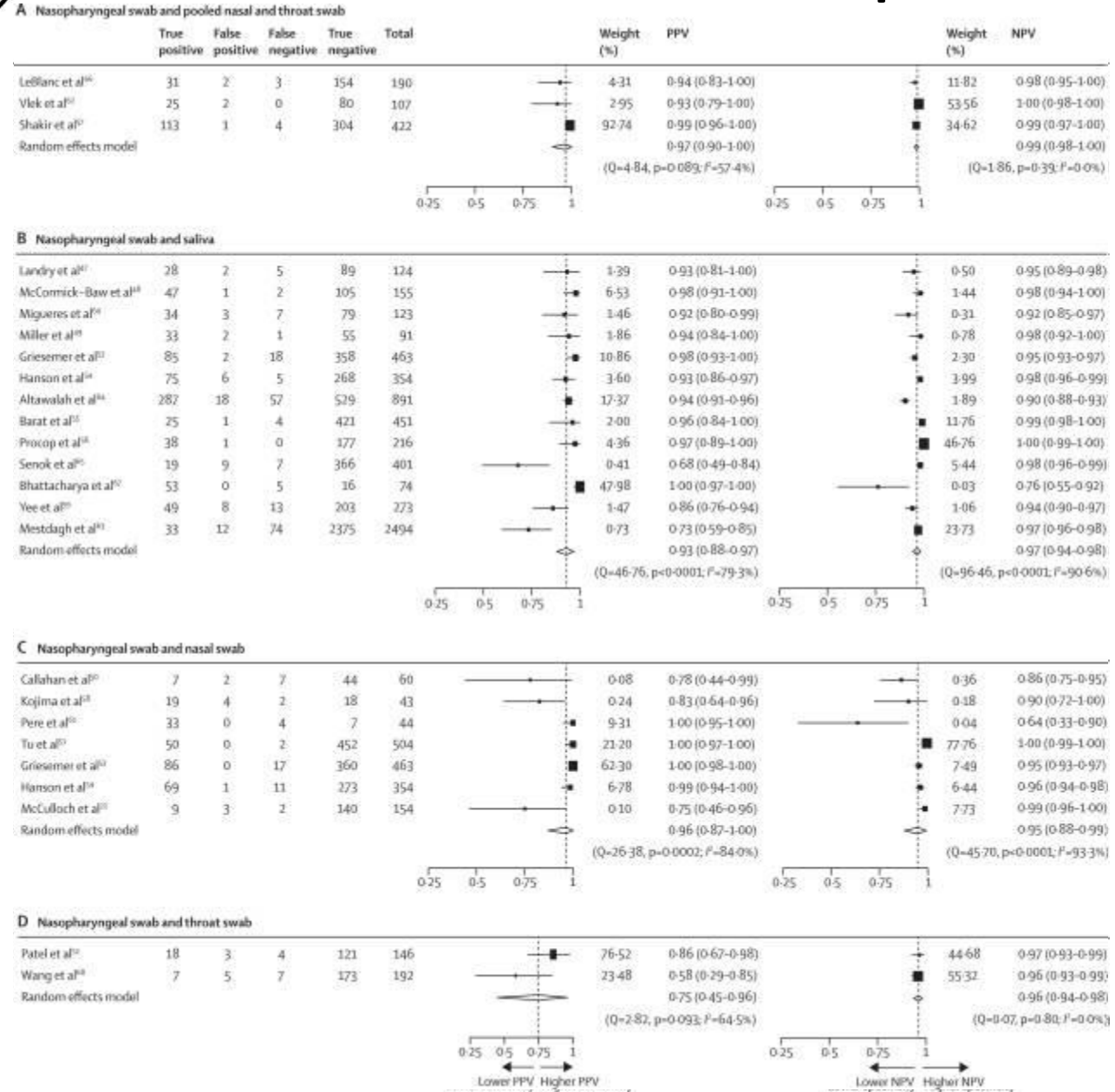






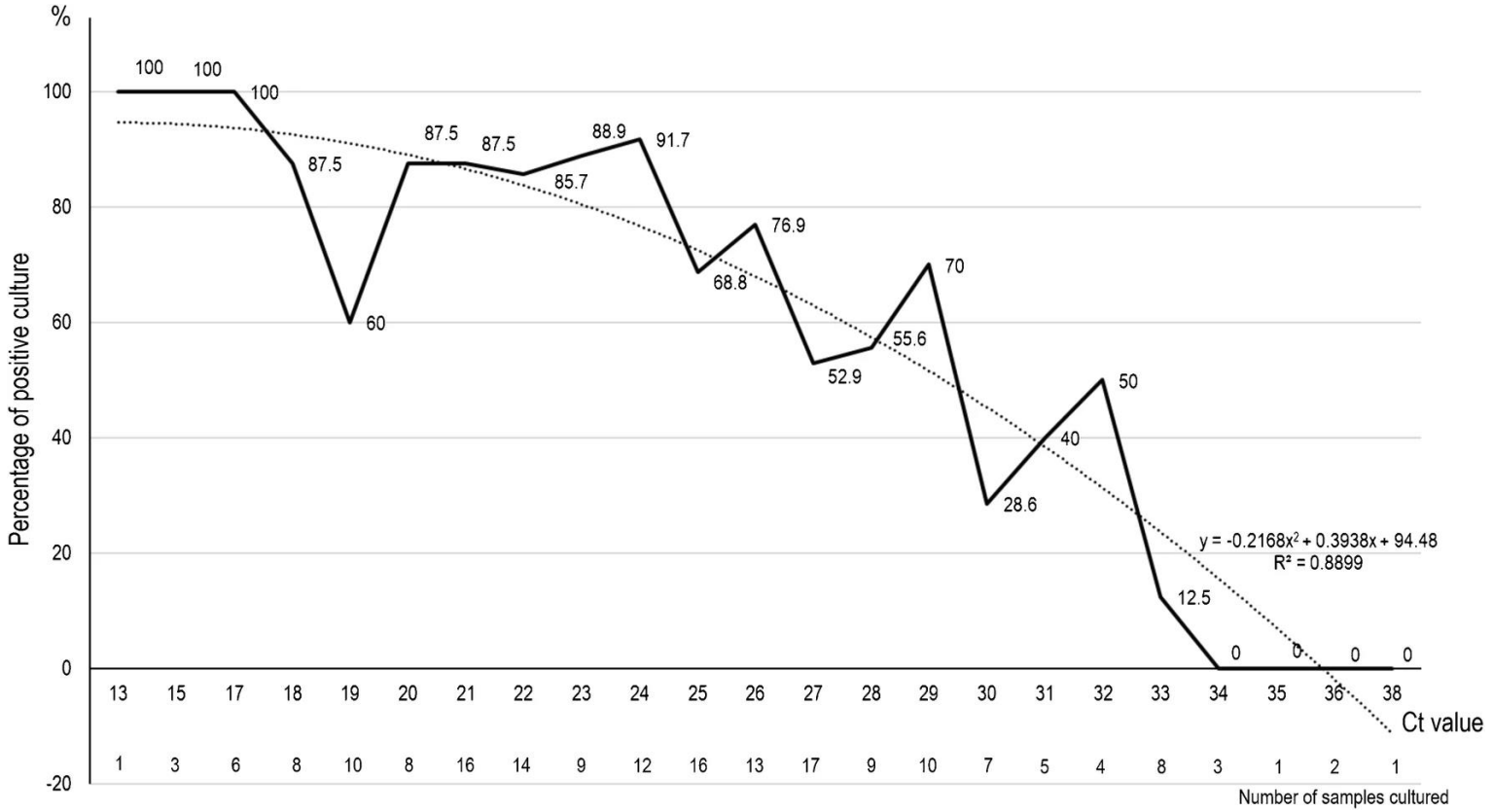
Lee et al. JAMA Intern Med. 2020;180(11):1447-1452.

SARS-CoV-2 virusno opterećenje



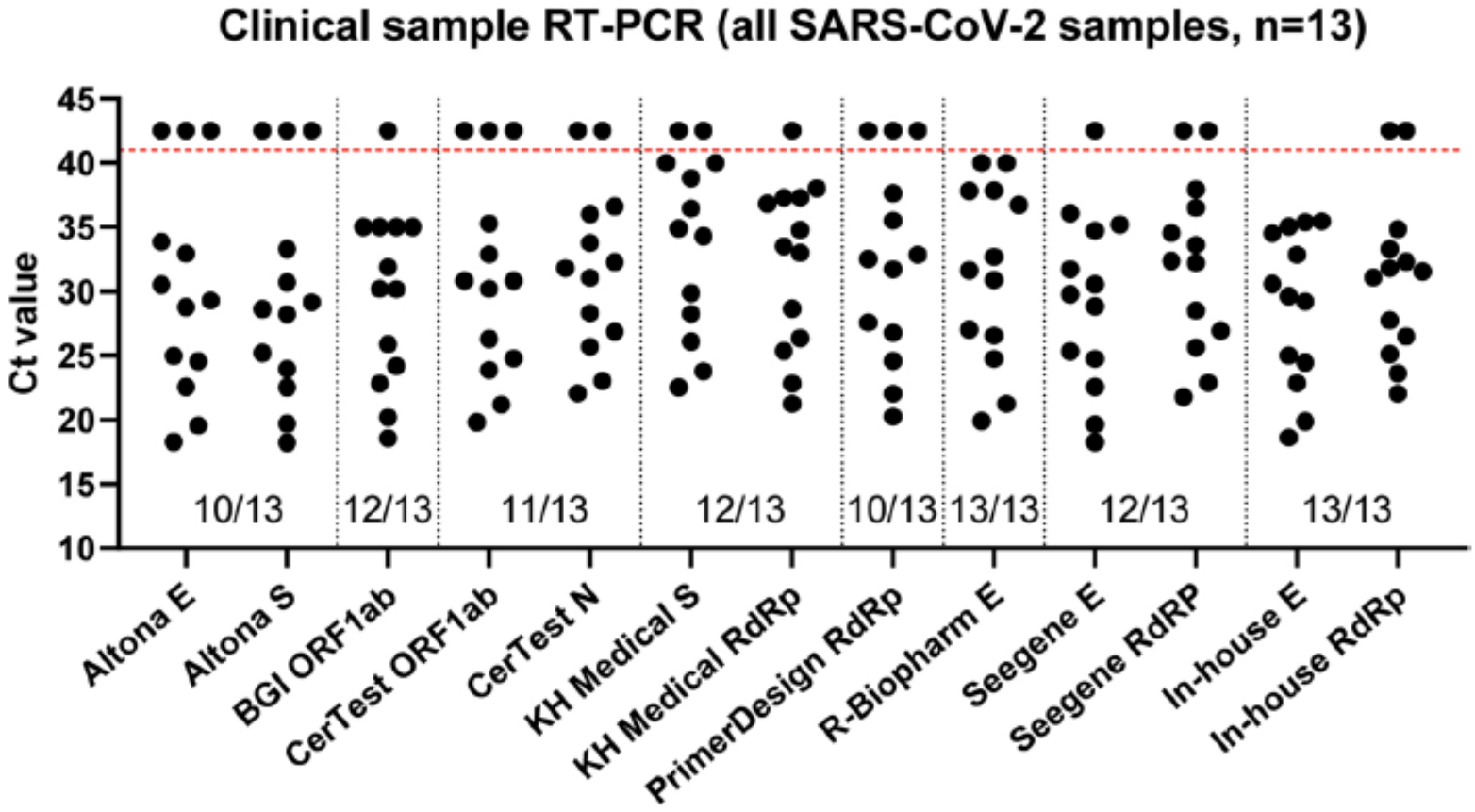
Yung Tsang et al. Lancet Infect Dis. 2021 Apr 12;S1473-3099(21)00146-8

SARS-CoV-2 virusno opterećenje



La Scola et al. Eur J Clin Microbiol Infect Dis. 2020 Jun;39(6):1059-1061

SARS-CoV-2 virusno opterećenje



van Kasteren et al. J Clin Virol. 2020 Jul;128:104412

SARS-CoV-2 virusno opterećenje

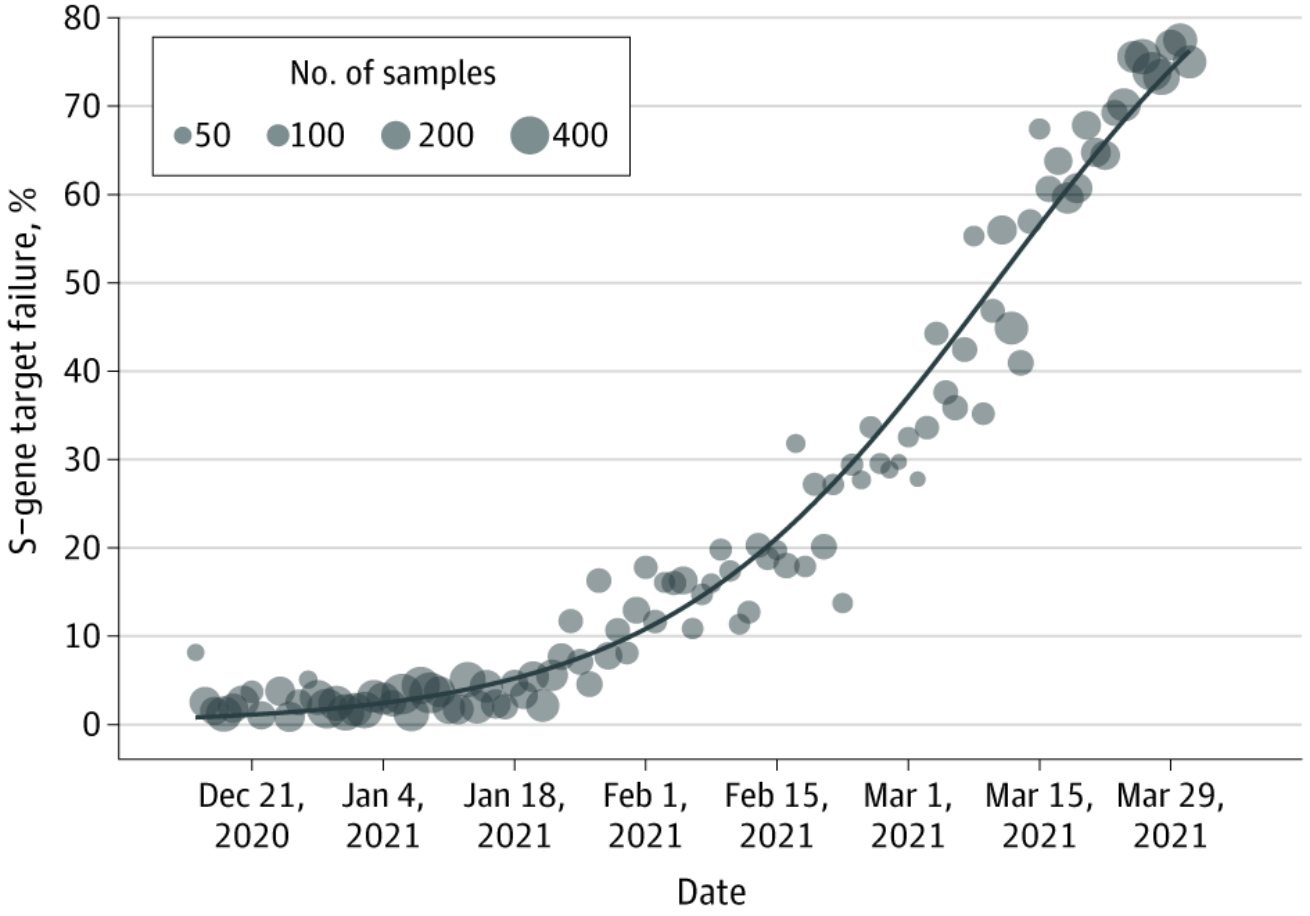
Panel 2	Ref	20	28	Influenca B	RSV	30	34	neg kontrola	21	Influenca A	31	26
Ustanova 1	**Rezultat**	poz	poz	neg	neg	poz	neg	neg	poz	neg	neg	poz
	gen E	24,5	33,5	-	-	34,5	38,7	-	25,8	-	-	29,2
	gen RDRp	26,3	34,5	-	-	35,3	-	-	27,4	-	-	32
Ustanova 2	**Rezultat**	poz	neod.	neg	neg	neod.	neg	neg	poz	neg	neod.	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

SARS-CoV-2 virusno opterećenje

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

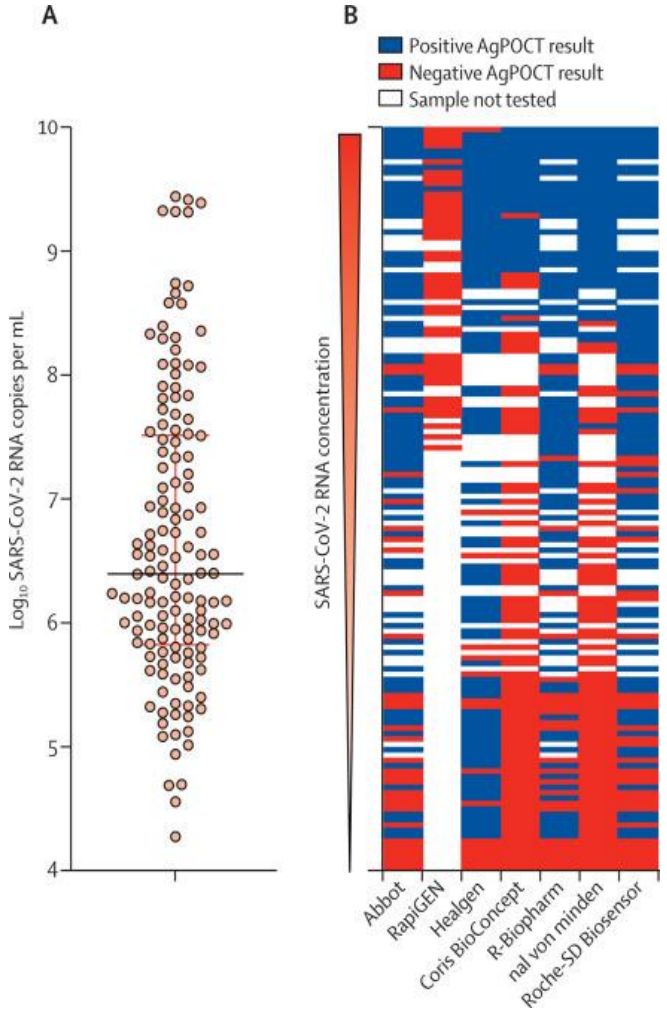
Barkod Hanks + Gondong štapić	Rez Hanks + Gondong štapić	Barkod Gongdong mediji + iClean štapić	Rez Gongdong mediji + iClean štapić	Razlika
0704*210	22,6	0704*322	27,9	5,3
0704*211	21,4	0704*327	31,7	10,3
0704*241	23,8	0704*318	26,4	2,6
0704*310	19,3	0704*433	26,1	6,8
0704*352	35,1	0704*435	37	1,9
0704*448	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

SARS-CoV-2 virusno opterećenje



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

Antigenski testovi



Corman et al. Lancet Microbe 2021 Apr 7

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

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

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

Varijante
VoC – Variants of Concern

WHO label	Lineage + additional mutations	Country first detected (community)	Spike mutations of interest	Year and month first detected	Evidence for impact on transmissibility	Evidence for impact on immunity	Evidence for impact on severity	Transmission in EU/EEA
Alpha	**B.1.1.7**	United Kingdom	N501Y, D614G, P681H	September 2020	Yes (v) [1]	No	Yes (v) [3, 4]	Dominating
	B.1.1.7+ E484K	United Kingdom	E484K, N501Y, D614G, P681H	December 2020	Yes (v) [1]	Neutralisation (v) [2, 5]	Yes (v) [3]	Outbreaks
Beta	**B.1.351**	South Africa	K417N, E484K, N501Y, D614G, A701V	September 2020	Yes (v) [6]	Escape (v) [7, 8]	Yes (v) [4, 9]	Community
Gamma	**P.1**	Brazil	K417T, E484K, N501Y, D614G, H655Y	December 2020	Yes (v) [10]	Neutralisation (v) [11]	Yes (v) [4]	Community
Delta	**B.1.617.2**	India	L452R, T478K, D614G, P681R	December 2020	Yes (v) [12-14]	Escape (v) [15]		Community

https://www.ecdc.europa.eu/en/covid-19/variants-concern

Varijante
VoI – Variants of Interest

WHO label	Lineage + additional mutations	Country first detected (community)	Spike mutations of interest	Year and month first detected	Evidence for impact on transmissibility	Evidence for impact on immunity	Evidence for impact on severity	Transmission in EU/EEA
Eta	**B.1.525**	Nigeria	E484K, D614G, Q677H	December 2020		Neutralisation (m) [5]		Community
Epsilon	**B.1.427/B.1.429**	USA	L452R, D614G	September 2020	Unclear [12]	Neutralisation (v) [12]		Sporadic/Travel
Theta	**P.3**	The Philippines	E484K, N501Y, D614G, P681H	January 2021	Yes (m) [1]	Neutralisation (m) [5]		Sporadic/Travel
	B.1.616	France	V483A, D614G, H655Y, G669S	February 2021	Detection (c) [17]			Single outbreak
Kappa	**B.1.617.1**	India	L452R, E484Q, D614G, P681R	December 2020	Yes (v) [18]	Neutralisation (v) [15, 17]		Outbreaks
	B.1.620	Unclear (b)	S477N, E484K, D614G, P681H	February 2021		Neutralisation (m) [5, 14]		Outbreaks
	B.1.621	Colombia	R346K, E484K, N501Y, D614G, P681H	January 2021	Yes (m) [1]	Neutralisation (m) [5]		Sporadic/

https://www.ecdc.europa.eu/en/covid-19/variants-concern

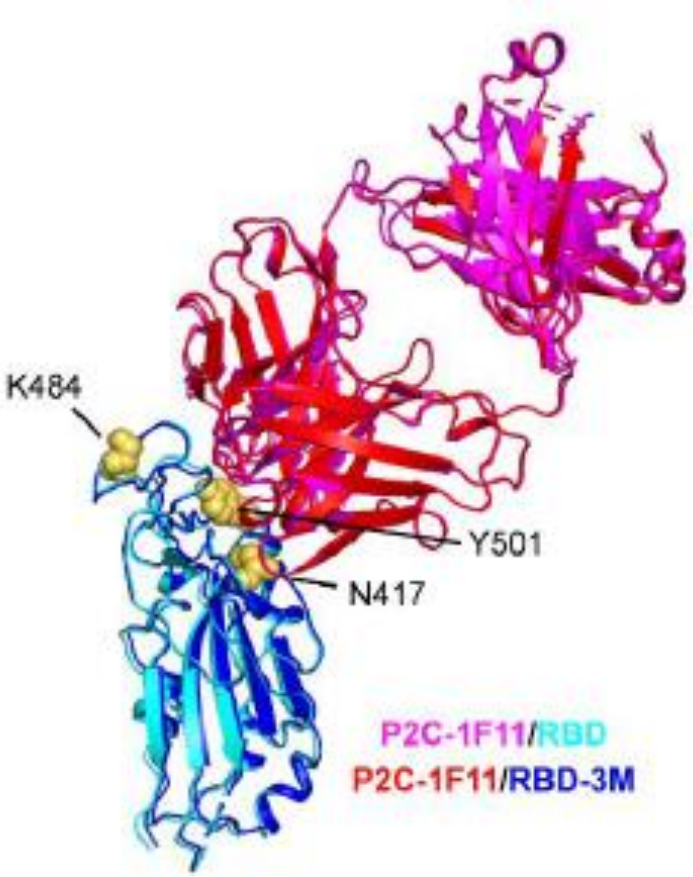
Varijante

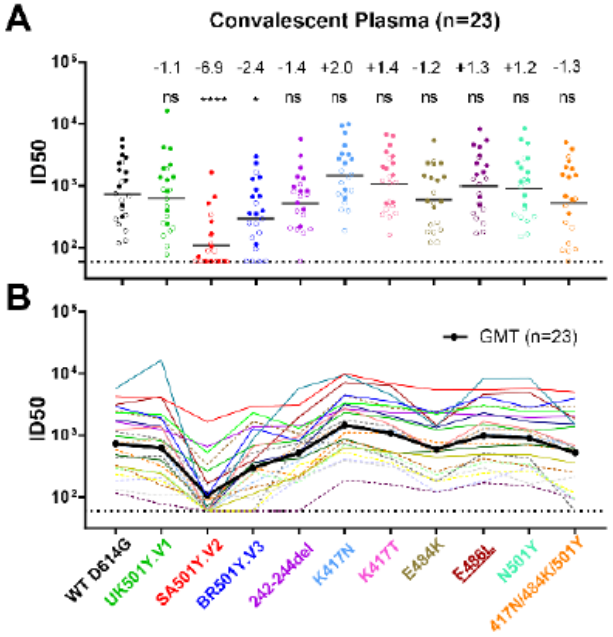
Vum – Variants under monitoring

WHO label	Lineage + additional mutations	Country first detected (community)	Spike mutations of interest	Year and month first detected	Evidence for impact on transmissibility	Evidence for impact on immunity	Evidence for impact on severity	Transmission in EU/EEA
	B.1.617.3	India	L452R, E484Q, D614G, P681R	February 2021	Yes (m) [1]	Neutralisation (m) [5, 12]		Not detected
	B.1.214.2	Unclear (b)	Q414K, N450K, ins214TDR, D614G	December 2020				Detected (a)
	A.23.1+ E484K	United Kingdom	V367F, E484K, Q613H	December 2020		Neutralisation (m) [5]		Detected (a)
	A.27	Unclear (b)	L452R, N501Y, A653V H655Y	December 2020	Yes (m) [1]	Neutralisation (m) [12]		Detected (a)
	A.28	Unclear (b)	E484K, N501T, H655Y	December 2020		Neutralisation (m) [5]		Detected (a)
	C.16	Unclear (b)	L452R, D614G	October 2020		Neutralisation (m) [5]		Detected (a)
	C.37	Peru	L452Q, F490S, D614G	December 2020				Detected (a)
	B.1.351+P384L	South Africa	P384L, K417N, E484K, N501Y, D614G, A701V	December 2020	Yes (v) [6]	Escape (v) [7, 8]	Unclear [9]	Detected (a)
	B.1.351+E516Q	Unclear (b)	K417N, E484K, N501Y, E516Q, D614G, A701V	January 2021	Yes (v) [6]	Escape (v) [7, 8]	Unclear [9]	Detected (a)
	B.1.1.7+L452R	United Kingdom	L452R, N501Y, D614G, P681H	January 2021	Yes (v) [1]	Neutralisation (m) [12]	Yes (v) [3]	Detected (a)
	B.1.1.7+S494P	United Kingdom	S494P, N501Y, D614G, P681H	January 2021	Yes (v) [1]	Neutralisation (m) [15]	Yes (v) [3]	Detected (a)
	C.36+L452R	Egypt	L452R, D614G, Q677H	December 2020		Neutralisation (m) [12]		Detected (a)
	AT.1	Russia	E484K, D614G, N679K, ins679GIAL	January 2021		Neutralisation (m) [5]		Detected (a)
Iota	**B.1.526**	USA	E484K, D614G, A701V	December 2020		Neutralisation (m) [5]		Detected (a)
	B.1.526.1	USA	L452R, D614G	October 2020		Neutralisation (m) [12]		Detected (a)
	B.1.526.2	USA	S477N, D614G	December 2020				Detected (a)
	B.1.1.318	Unclear (b)	E484K, D614G, P681H	January 2021		Neutralisation (m) [5]		Detected (a)
Zeta	**P.2**	Brazil	E484K, D614G	January 2021		Neutralisation (m) [5]		Detected (a)
	B.1.1.519	Mexico	T478K, D614G	November 2020		Neutralisation (m) [12]		Detected (a)
	AV.1	United Kingdom	N439K, E484K, D614G, P681H	March 2021		Neutralisation (m) [5]		Detected (a)
	P.1+P681H	Italy	D614G, E484K, H655Y, K417T, N501Y, P681H	February 2021		Unclear [24, 25]		

https://www.ecdc.europa.eu/en/covid-19/variants-concern

Varijante



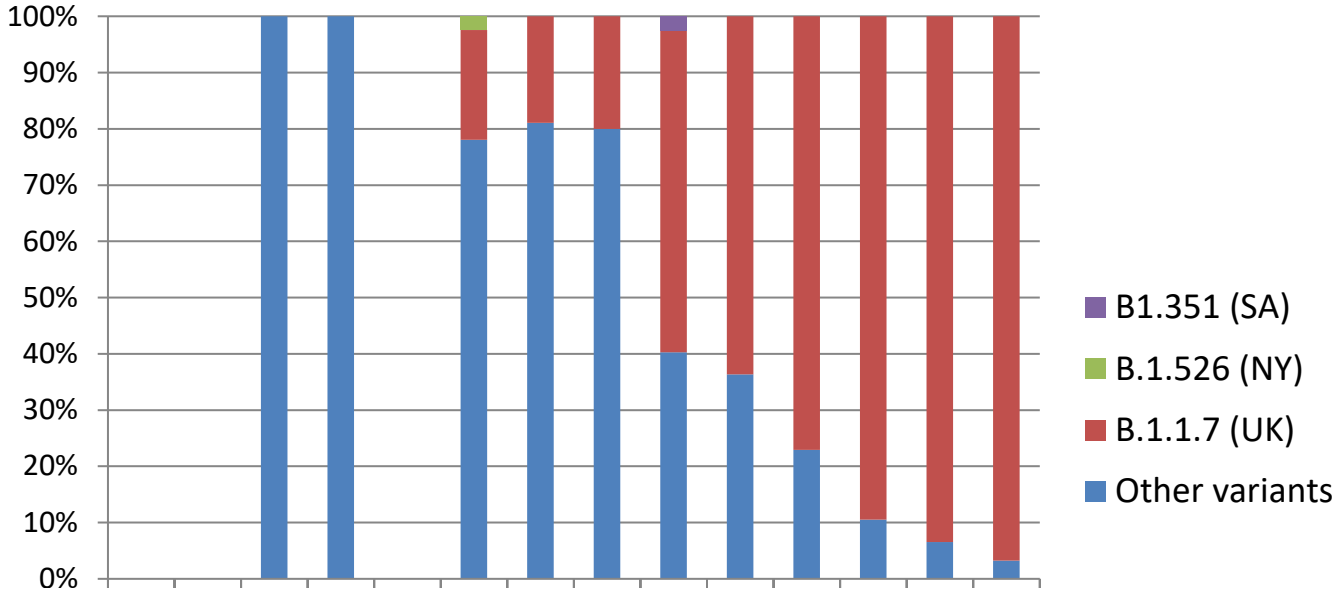


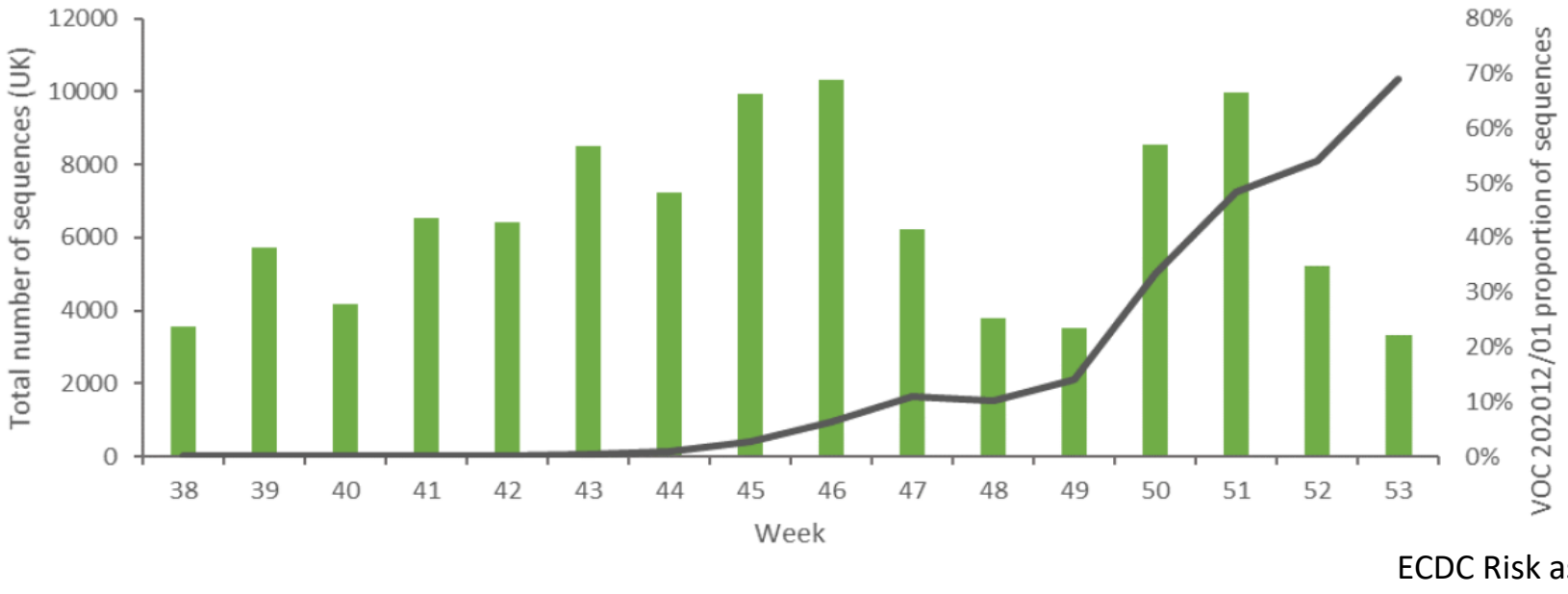
Fold changes in ID50	WT	Variants		NTD	RBD					
C-Plasma1	+1.0	-1.5	-27.6	-2.3	-3.6	+1.5	+1.2	-1.2	+1.5	-1.0	+1.4
C-Plasma2	+1.0	-1.1	-2.6	-1.4	-1.4	+2.3	+1.6	+1.3	+1.3	+1.3	+1.2
C-Plasma3	+1.0	-1.2	-3.7	-1.4	-1.2	+2.4	+1.9	+1.3	+1.6	+1.2	+1.4
C-Plasma4	+1.0	-1.3	-2.5	-1.2	-1.2	+1.6	+1.4	+1.4	+1.3	+1.2	+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, F486L, N501Y, 417N/484K/501Y

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

Varijante





ECDC Risk assessment published 21.01.21.

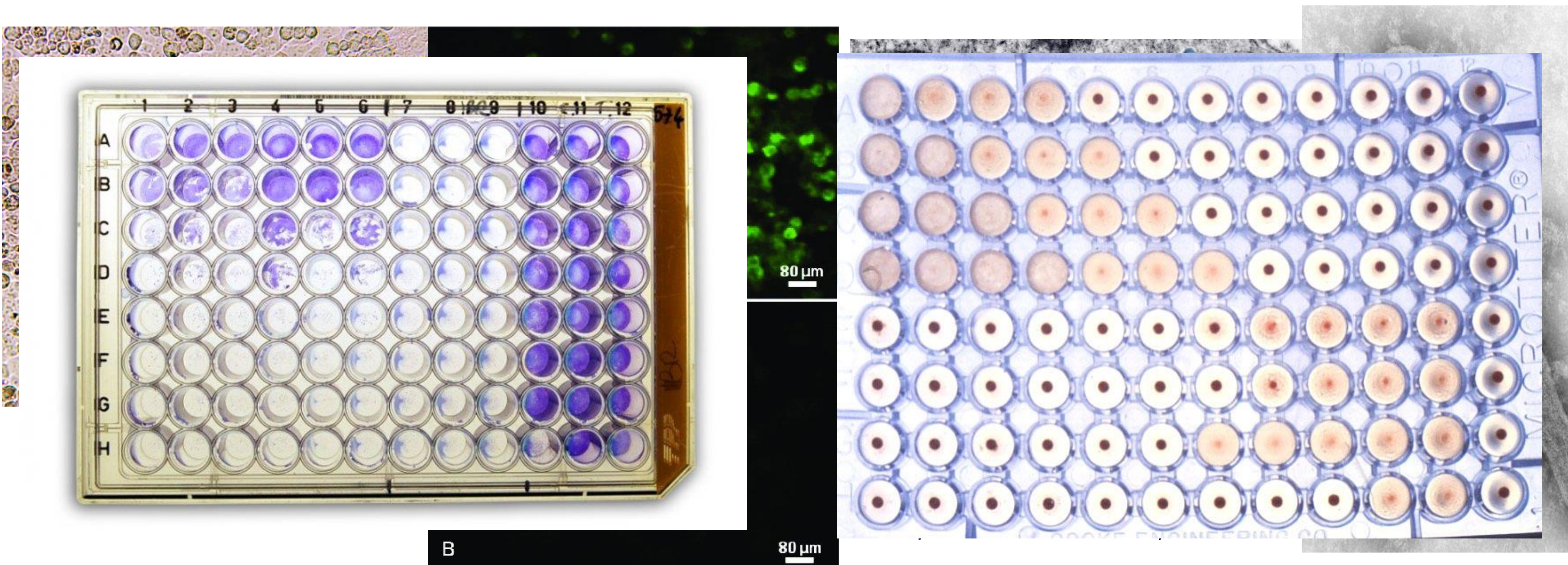
Otkrivanje novih virusa – klasična virusologija

Izolacija uzročnika:

Serološka karakterizacija uzročnika:

1. Inokulacija uzorka na stanice, oplođena jaja ili miševe
2. Opservacija u karakterizaci
    - CPE / smrt ploda / smrt miša
    - Imunofluorescencija / elektronska mikroskopija

1. Test neutralizacije
2. Test hemaglutinacije



CDC/ F.A. Murphy

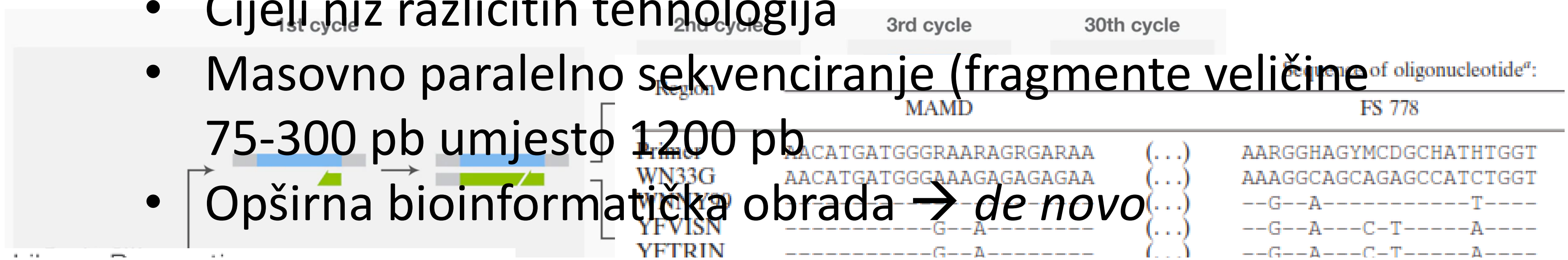
Martins et. al. Mem Inst Oswaldo Cruz. 2019. 17;114:e180332

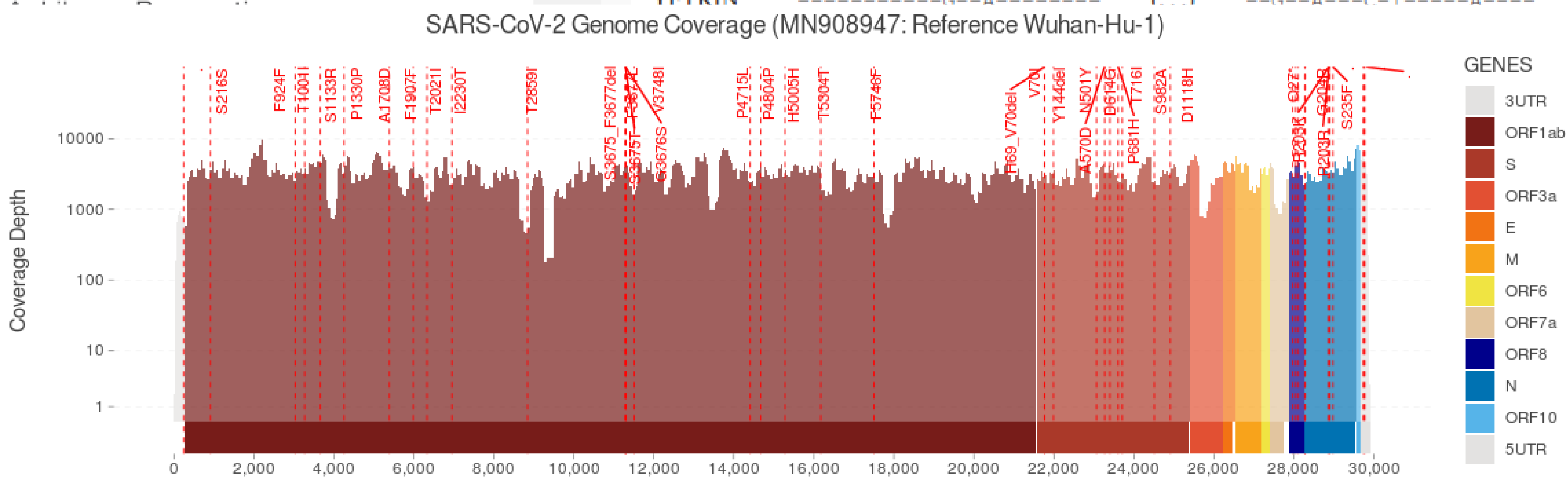
Otkrivanje novih virusa – molekularne metode

Pan-PCR: specifični PCR za cijelu grupu uzročnika

NGS: sekvenciranje nove generacije

- Cijeli niz različitih tehnologija
- Masovno paralelno sekvenciranje (fragmente veličine 75-300 pb umjesto 1200 pb
- Opširna bioinformatička obrada → *de novo*





Hvala na pažnji!

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