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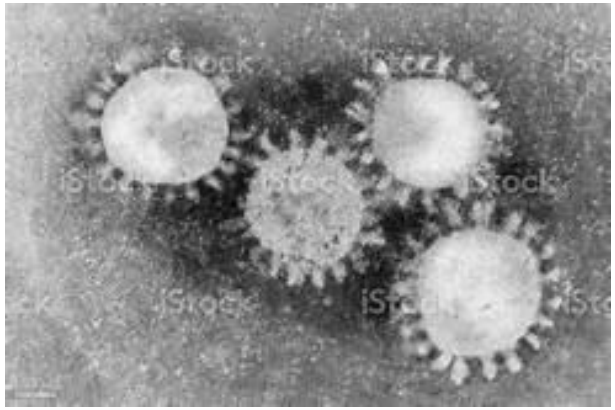
Origin and Evolution of SARS-COV-2



Relatore: PhD Vicenti Ilaria, ricercatrice

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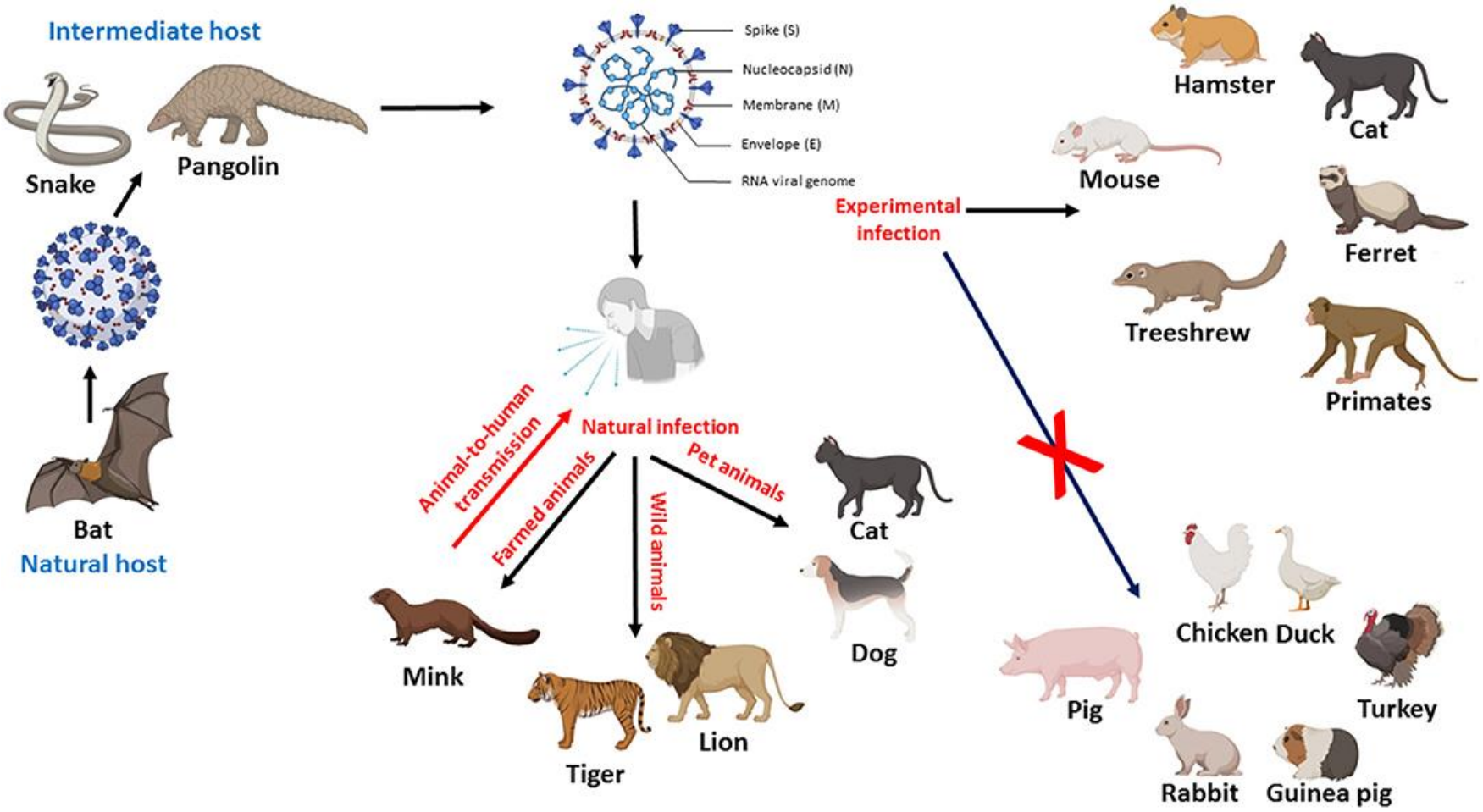
Coronavirus

- Long known as animal and human pathogens (first coronavirus discovered in 1931)
- High propensity for genetic recombination across host species boundaries (spillover)
- Persistent in reservoir hosts
- Increasing contact between humans and a variety of wild mammals provides abundant opportunities for inter-species transfer

SARS-CoV-2 host reservoir



It is essential to identify the potential virus reservoir to avoid repeated contacts among the species involved

Mahdy Mohamed et al., Frontiers in Medicine 2020

SARS-CoV-2 evolution

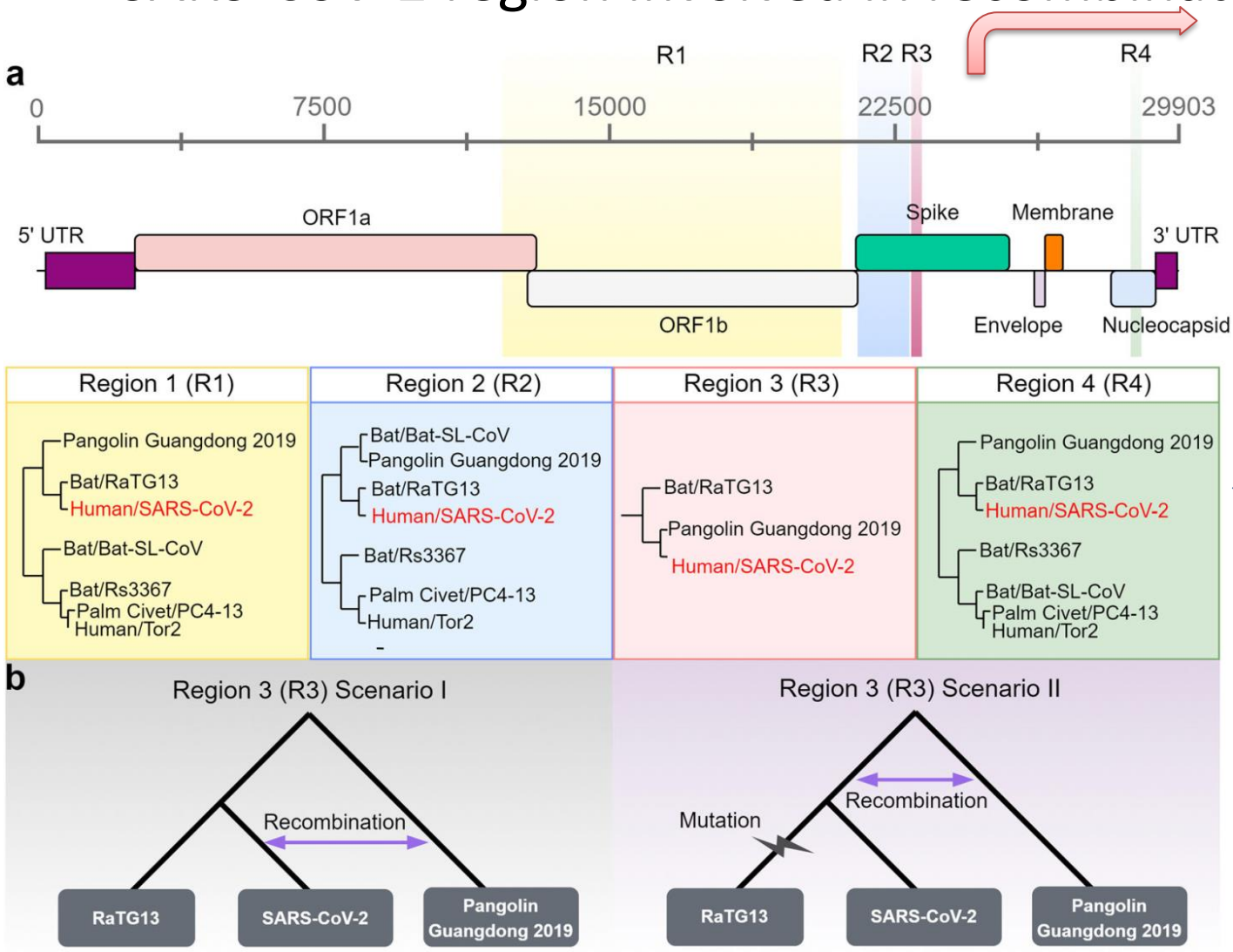
Table 2. SARS-CoV-2 genomic sequence similarity to SARS-like coronaviruses, SARS-CoV and MERS-CoV.

Coronavirus Strain	Genomic Sequence Similarity to SARS-CoV-2	Reference
SARS-like coronavirus bat-RaTG13	96.2%	[21]
SARS-like coronavirus bat-SL-CoVZC45	88%	[21]
SARS-like coronavirus bat-SL-CoVZXC21	88%	[21]
SARS-CoV	79%	[39]
MERS-CoV	51.8%	[40]

Sharma et al., Viruses 2021

- The closest SARS-CoV-2 relative is a coronavirus strain found in a bat sample from Yunnan Province, China, in 2013. This strain is referred to as "RaTG13" (horseshoe bat, Rhinolophus affinis) (*Boni et al, Nature Microbiology 2020*).
- The divergence distance increases in the Receptor Binding Domain:
  - The spike is a mosaic of different recombination events

SARS-CoV-2 region involved in recombination events



R3 includes the variable loop region of the S protein and 6 residues of RBD. It shares the closest similarity with the pangolin coronavirus strain

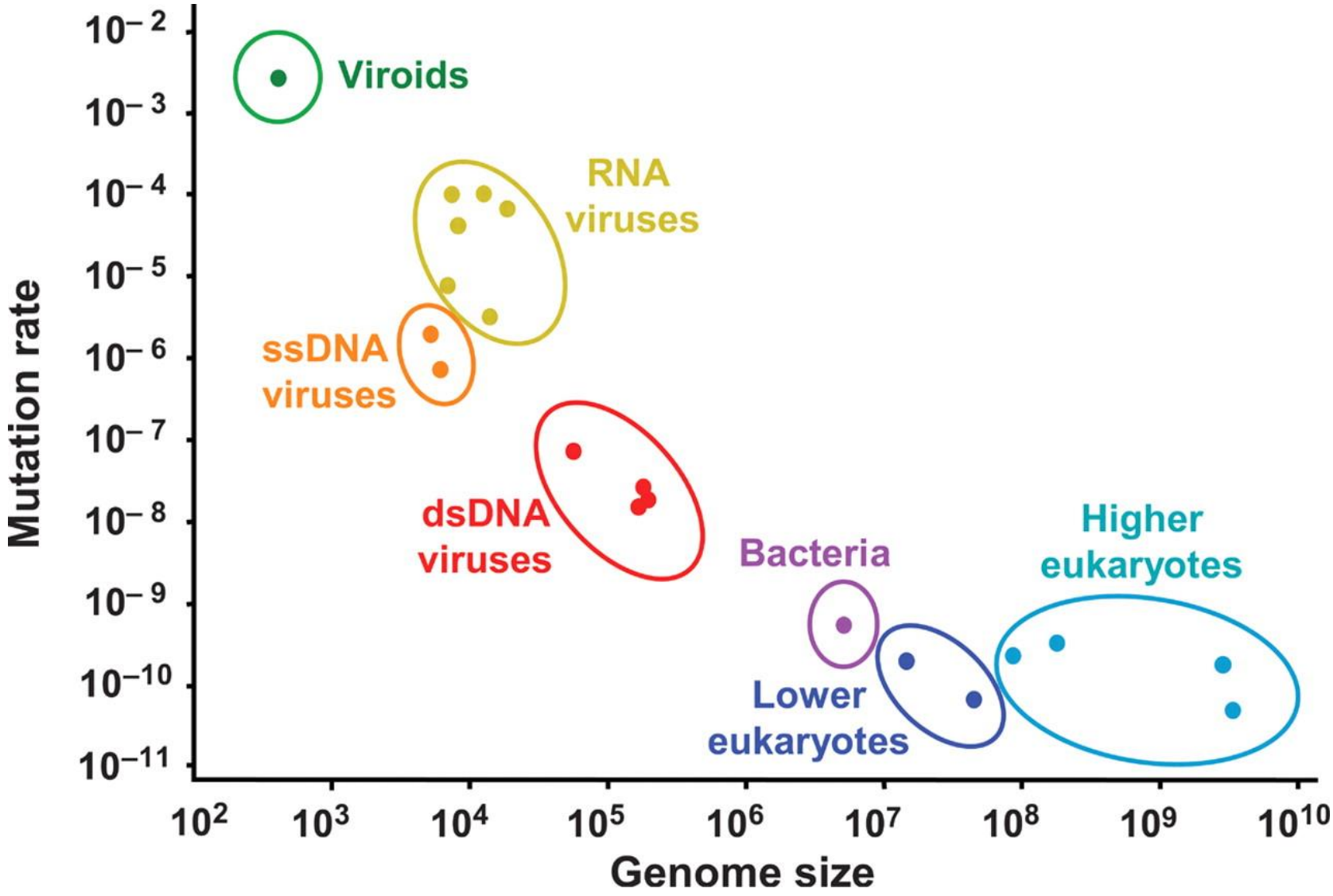
Regions 1, 2 and 4 are closely related to the bat coronavirus RaTG13

Singh et al, Exp Mol Med 2021

Scenario 1:
1) SARS-CoV-2 and RaTG13 divergence
2) SARS-CoV-2/Pangolin Guangdong 2019 recombination

Scenario 2:
1) Common ancestral lineage of SARS-CoV-2/RaTG13/Pangolin Guangdong 2019 lineage recombination
2) accumulation of mutations in the RaTG13 lineage.

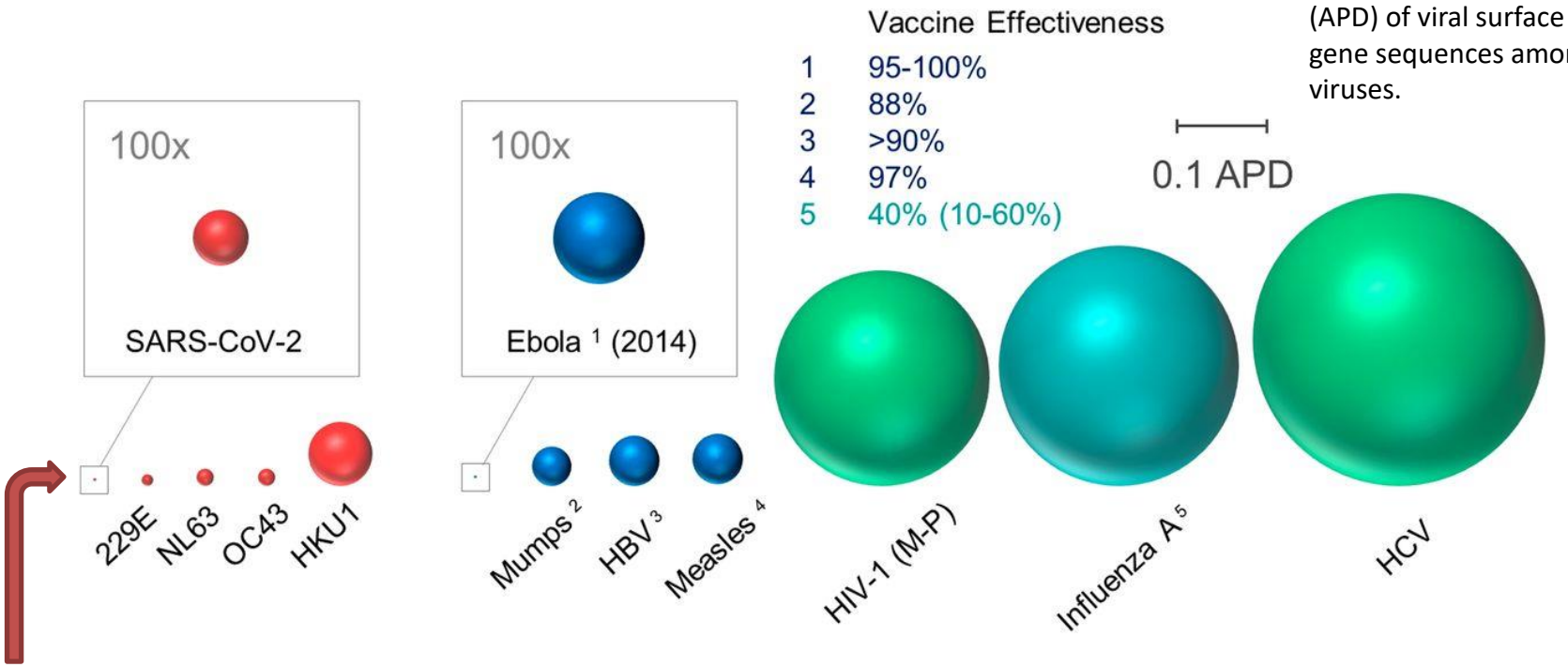
Spontaneous mutations rate



Gago S et al., Science 2009

Comparative genetic diversity among surface glycoproteins

As indicated by the scale bar, sphere radius reflects average pairwise distances
(APD) of viral surface glycoprotein gene sequences among different viruses.

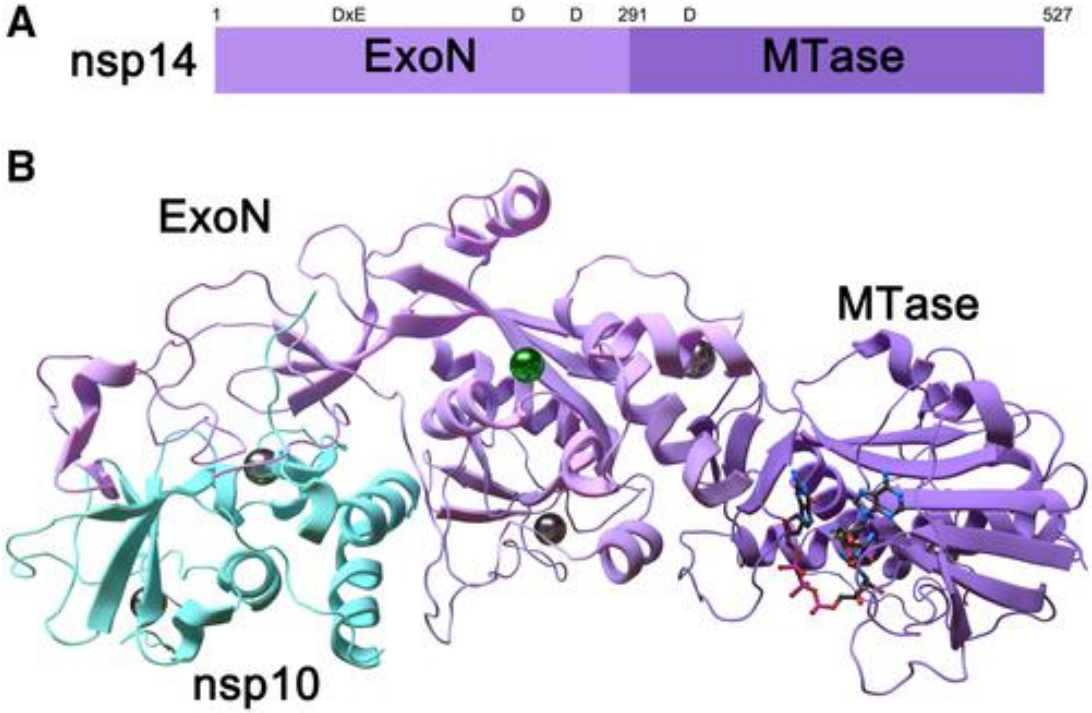


Rausch et al., PNAS 2020 doi: 10.1073/pnas.2017726117

low rate of antigenic variation

The evolutionary rate of SARS- CoV-2 has been estimated to be between 0.0004 and 0.002 mutations per nucleotide per year

Additional mechanisms of error correction: RNA proofreading



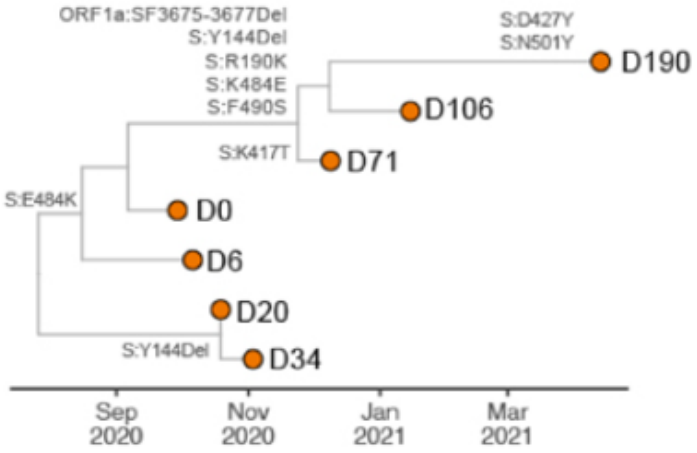
- Nsp14 is a DEDD exonucleases with proofreading activity, initially discovered in SARS-CoV
- Knockout mutants of SARS-CoV were shown to accumulate a high number of mutations (Saramago M et al., The FEBS Journal 2021).
- Nsp14 reduces replication error rate by about 15- fold to 20- fold in vitro, resulting in an in vivo viral mutation rate about 10- fold lower than that of influenza (Tao et al., Nature Review 2021)
- Inactivation of ExoN results in decreased recombination frequency in vitro (Gribble et al., PloS Pathogens 2021)

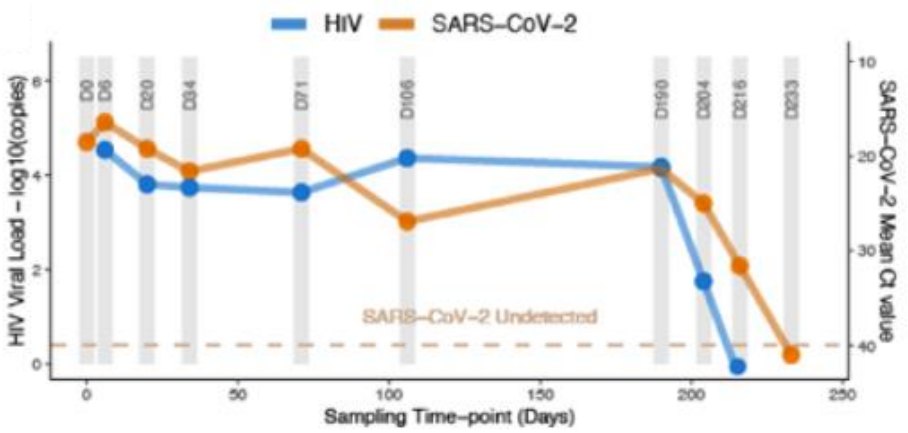
SARS-CoV-2 Evolution during pandemic

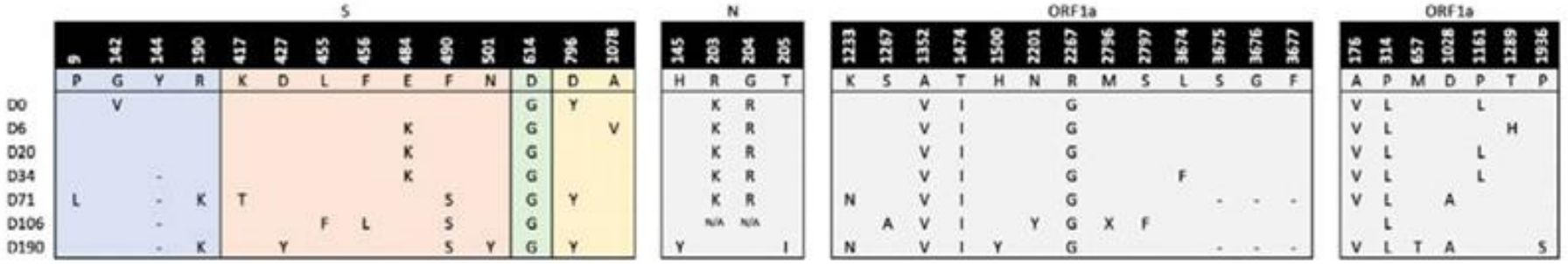
Factors driving SARS-CoV-2 evolution despite a relatively low mutation rate:

- Extremely large **population size** and prolonged **duration** of the pandemic
- Generation of **insertion and/or deletions** which are not subject to proofreading activity (*McCarthy et al., Science 2021*)
- Host-mediated **RNA editing** by APOBEC and ADAR enzymes (C to U changes in specific dinucleotide contexts)
- **Recombination** events and intra-host evolution

Persistent infection allows intra-host viral evolution







The E484K mutation in the spike RBD emerged very early in the course of infection, At day 34, the Y144 deletion emerged and persisted up to day 106. As infection persisted, the E484K mutation was replaced by mutations at other sites in the RBD

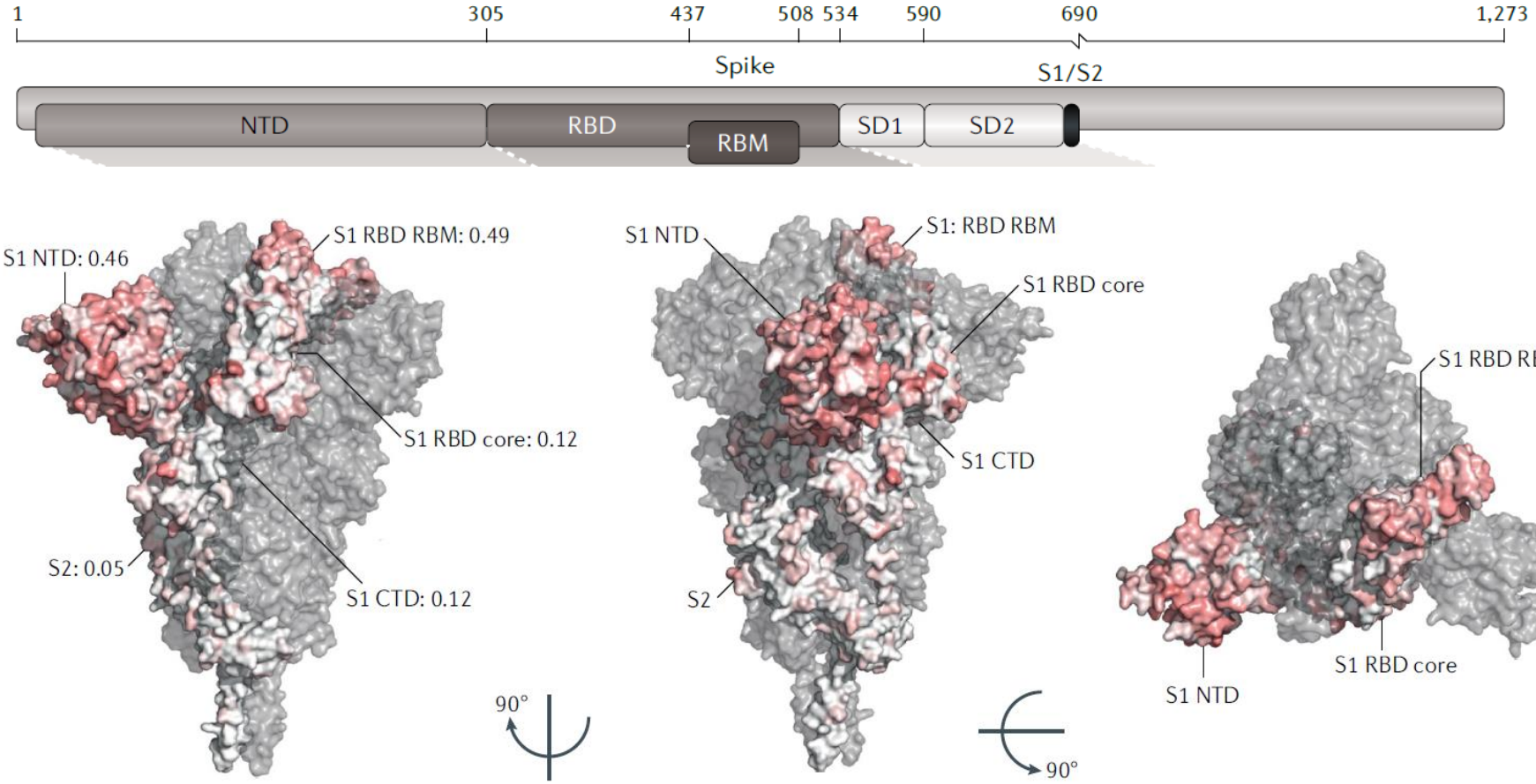
***Karim et al., medRxiv**, Jun 2021*

Variant: VOC, VOI and VUM

- VARIANT: A viral genome containing one or more not-synonymous mutations, mostly detected in the spike gene
- VOC: Variants that have spread widely and displayed evidence for being **more transmissible**, causing **more severe disease** and/or **reducing neutralization by antibodies** generated during previous infection or vaccination
- VOI: Variants that have spread less widely but contain mutations similar to those present within VOCs
- VUM: Variants with genetic changes that are suspected to affect virus characteristics. Evidence of phenotypic or epidemiological impact is currently unclear, requiring enhanced monitoring.

https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/

Spike structure



- The SARS- CoV-2 spike protein is a trimeric glycoprotein responsible for virus entry into host cells
- Attachment domain (S1), Fusion domain (S2), Receptor Binding domain (RBD; 328aa), Receptor binding motif (RBM; 20aa)
- RBD alternates between a closed/down position and an open/up position (bound to ACE-2)
- Variability: RBM>RBD>S1>S2

Tao et al. Nature Reviews 2021

VOC (Last update by WHO 23/02/2022)

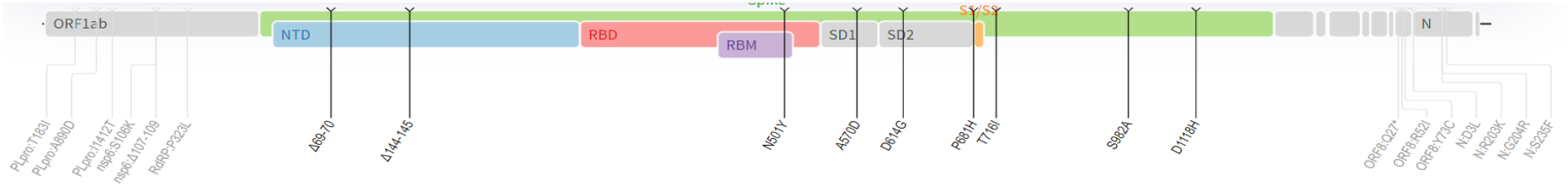
WHO label	Pango lineage*	Nextstrain clade	Additional amino acid changes monitored§	Earliest documented samples	Date of designation	ECDC°	CDC°
Alpha	B.1.1.7	20I (V1)	+S:484K +S:452R	United Kingdom, Sep-2020	18-Dec-2020	De-escalated	VBM
Beta	B.1.351	20H (V2)	+S:L18F	South Africa, May-2020	18-Dec-2020	VOC	VBM
Gamma	P.1	20J (V3)	+S:681H	Brazil, Nov-2020	11-Jan-2021	VOC	VBM
Delta	B.1.617.2	21A, 21I, 21J	+S:417N +S:484K	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021	VOC	VOC
Omicron	B.1.1.529	21K, 21L 21M	+S:R346K	Multiple countries, Nov-2021	VUM: 24-Nov-2021 VOC: 26-Nov-2021	VOC	VOC

*Phylogenetic Assignment of Named Global Outbreak (PANGO). Alphabetical prefix and a suffix containing up to 3 numbers separated by periods indicating sub- lineages. (https://cov-lineages.org/index.html#cite)
+ Nextstrain. The first numbers represent the year of variant emergence followed by a letter assigned when the variant reach the 20% of worldwide prevalence
§ Only found in a subset of sequences
° ECDC and CDC designation as VOC, De-Escalated variants (ECDC) or VBM (Variant Being Monitored)

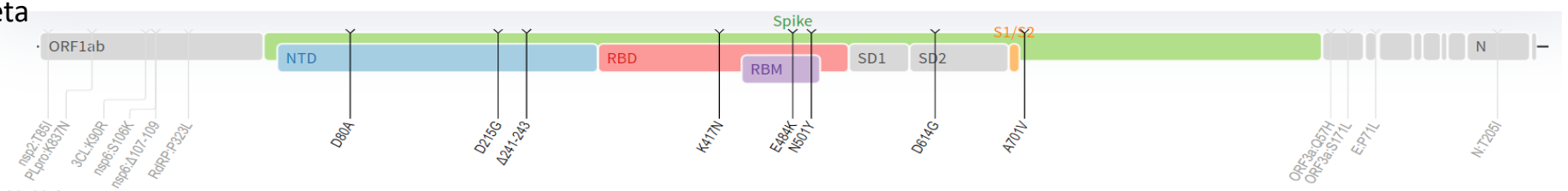
Adapted by WHO https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/

Mutational pattern of VOCs

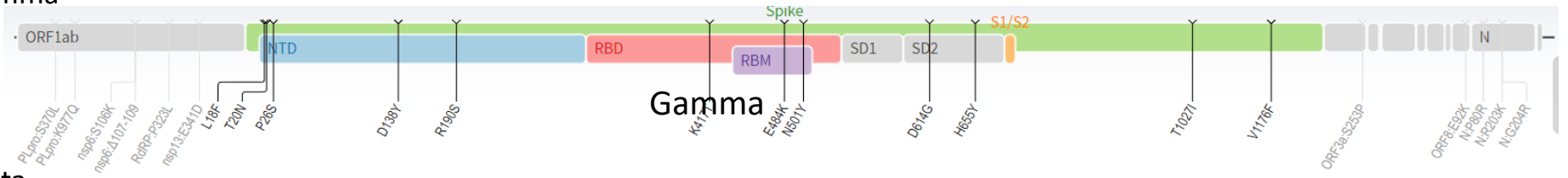
Alpha



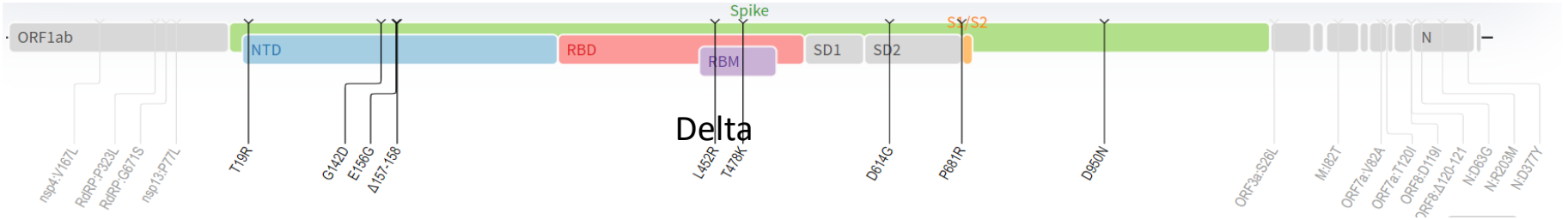
Beta



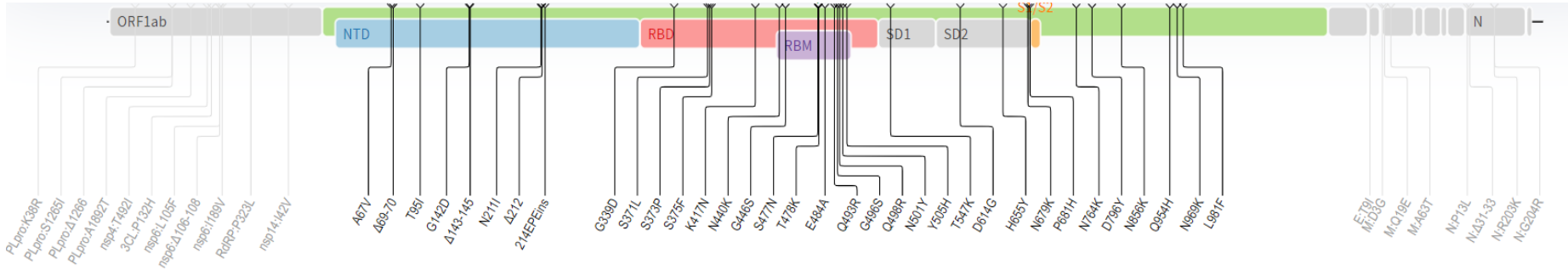
Gamma



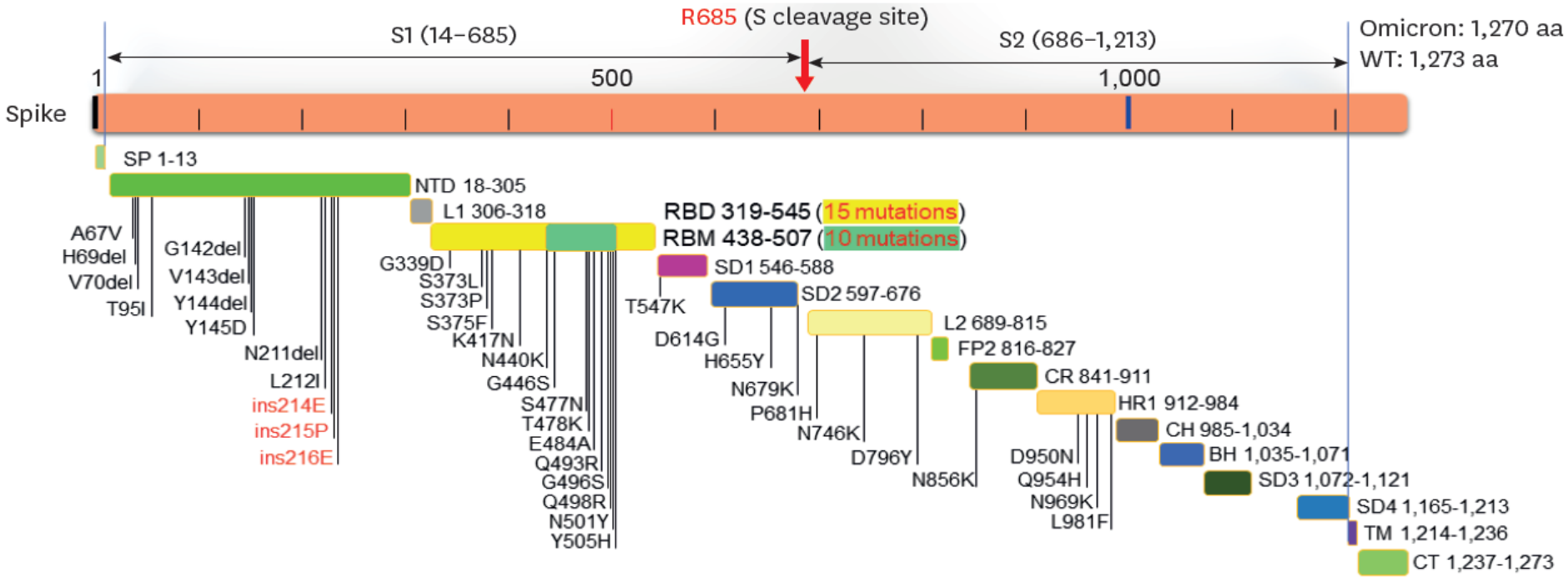
Delta



Omicron



Mutational pattern of Omicron variant in detail



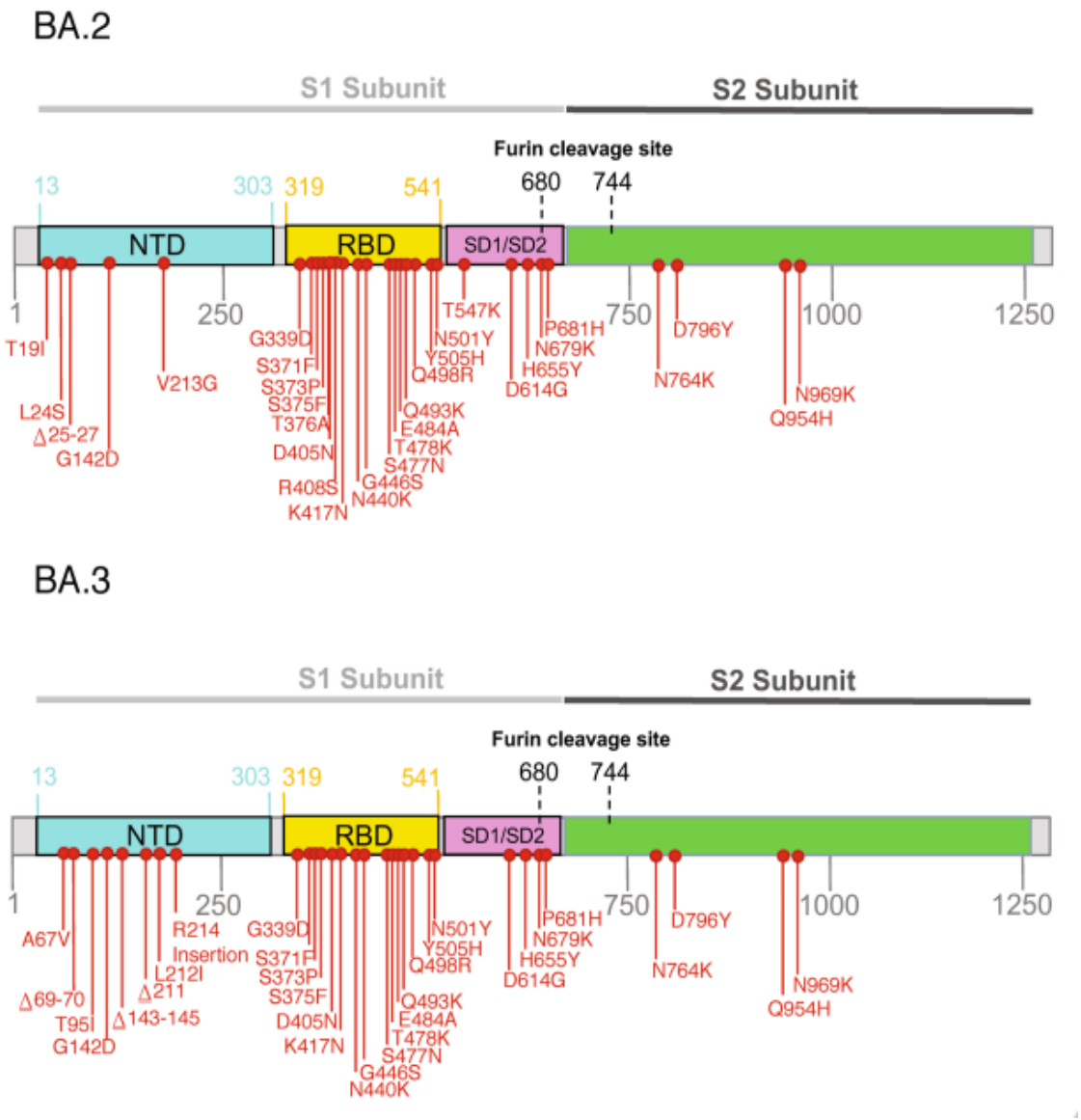
Omicron (S. Africa) – 39 amino acid residues (27 new mutation sites)

A67V, H69del, V70del, T95I, G142del, V143del, Y144del, Y145D, N211del, L212I, ins214E, ins215P, ins216E, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

In red are indicated the new mutations; the mutations located in the RBD (15) and RBM (5) are underlined in yellow and green respectively

Kim et al., 2022 Immune Network

Further genetic evolution of Omicron: BA.2 (21L) and BA.3



- **BA.2**: accumulation (T19I, L24S, P25-P26-A27 del, V213G, T376A, D405N, and R408S) or loss (V67A, H69-V70del, T95I, V143-Y144-Y145del, N211del, Ins214EPE, G446S, G496S, T547K, N856K, and L981F) of several mutations
- **BA.3** shared the entire mutational set with the BA.1, with the addition of the RBD mutation D405N, and the loss of G496S, N856K, and L981F

Viana et al., Nature 2022

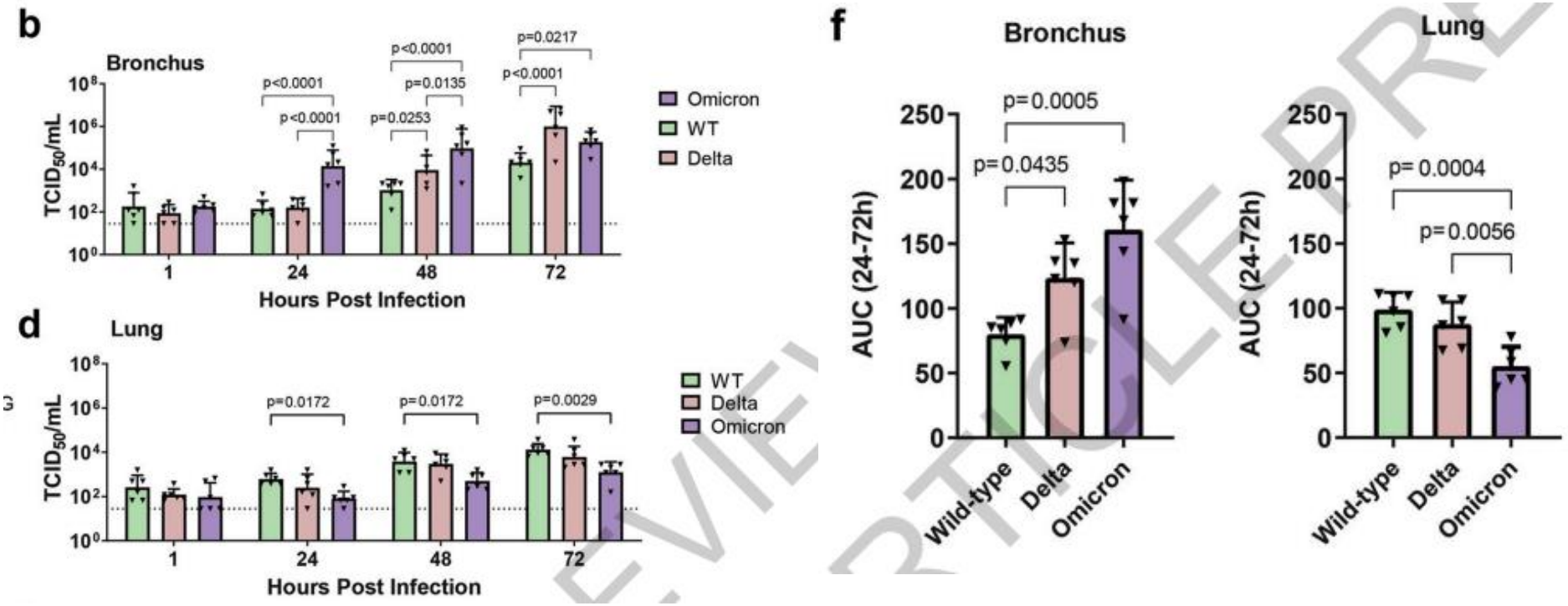
Characteristics of the main variants of SARS-CoV-2

WHO label	Spike protein substitutions	Increased transmissibility	Increased infectivity	Increased disease severity	mAbs escape	Immune escape*
Alpha	ΔH69, ΔV70, ΔY144, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)	**Yes**	**Yes**	**Yes**	**No**	**No**
Beta	D80A, D215G, Δ241, Δ242, Δ243, V367F, P384L, R408I, K417N, E484K, N501Y, D614G, A701V	**Yes**	**Yes**	**No**	**No**	**Yes**
Gamma	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	**Yes**	**No**	**No**	**Yes (El Lilly combination)**	**No**
Delta	T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, D614G, P681R, D950N	**Yes**	**Yes**	**Yes**	**No**	**Intermediate**
Omicron	A67V, ΔH69, ΔV70, T95I, G142D, ΔV143, ΔY144, ΔY145, ΔN211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, L981F, N856K	**Yes**	**Yes**	**No**	**Yes (high for El Lilly and Regeneron combination, medium for Sotrovimab)**	**Yes**

* Intended as immune escape from neutralizing antibodies. Mutations at T cell epitopes have been observed but not shown to impact the cytotoxic T lymphocyte (CTL) response

Adapted by Jiang et al., Frontiers in Medicine 2022

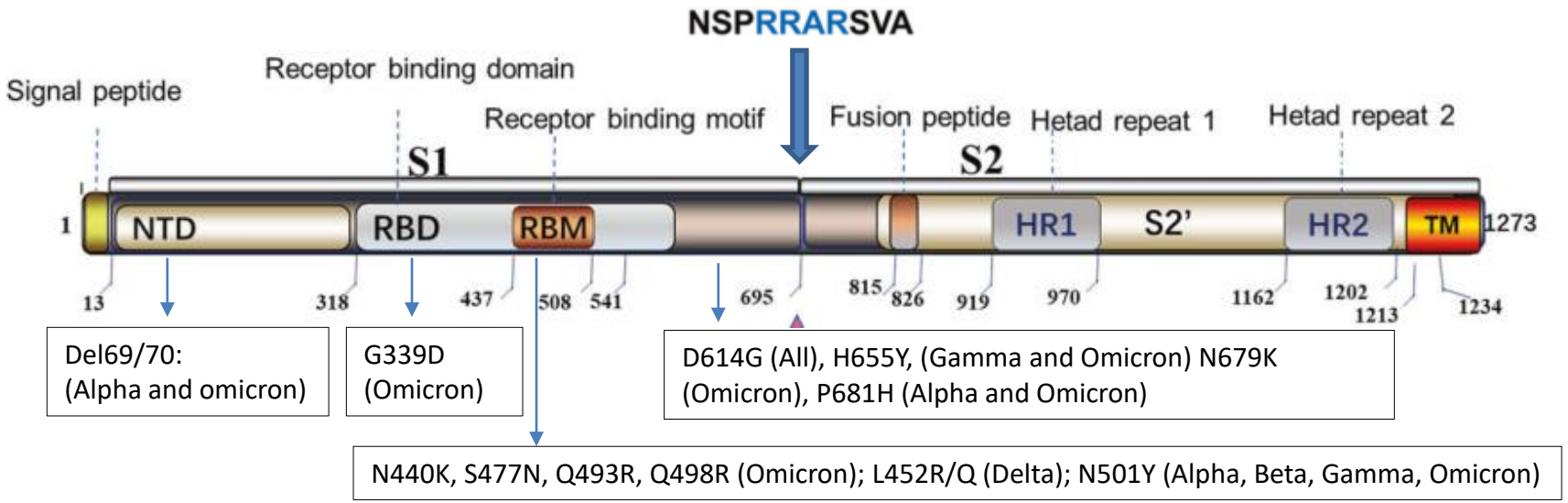
Omicron replicated faster than all other SARS-CoV-2 in the bronchus but less efficiently in the lung parenchyma



Viral replication kinetics of SARS-CoV-2 variants in ex vivo cultures of human respiratory tract. Viral titers from **b** to **d** are depicted as area under the curve (AUC) in **f**

Hui et al., Nature 2022

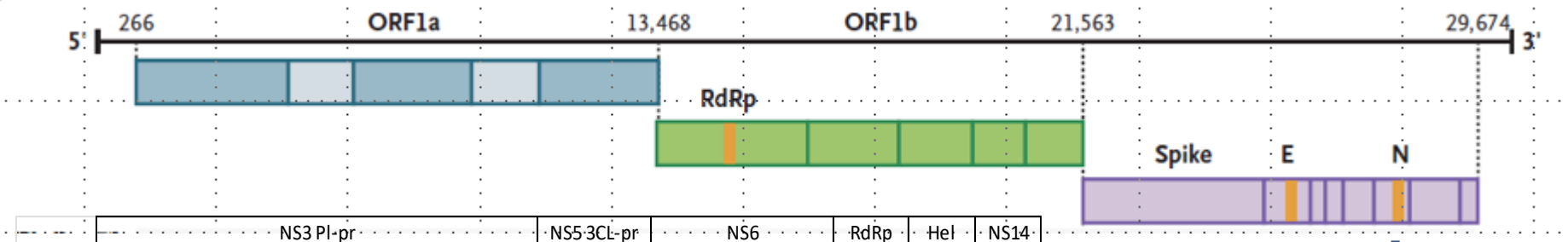
Mutations conferring increased infectivity and transmissibility



- Increased binding affinity to ACE-2 (e.g., G339D, Q493R, N501Y)
  - Synergistic role of some mutations (e.g. Q498R + N501Y gives 4-fold increase with respect to N501Y alone)
- Enhanced virus release (e.g., N440K)
- Increased spike cleavage (S1/S2 junction; e.g., H655Y)

Adapted by Alkhatib et al., 2021 Microbiology Spectrum

Non spike mutations conferring increasing transmissibility



	NS3 Pl-pr											NS5 3CL-pr		NS6					RdRp		Hel			NS14	
wt	K	T	S	T	K	A	K	S	L	I	A	K	P	T	S	G	F	I	P	G	P	E	M	I	A
Variants	38	183	370	749	837	890	977	1265	1266	1412	1892	90	132	77	106	107	108	189	323	671				42	394
Alpha		I				D				T					DEL	DEL	DEL		L						
Beta					N							R			DEL	DEL	DEL		L						
Gamma			L				Q								DEL	DEL	DEL		L			D			
Delta														A					L	S	L		I		V
Omicron	R							DEL	I		T		H		DEL	DEL	DEL	V	L					V	

	N											
wt	D	P	E	R	S	D	P	R	G	T	S	D
Variants	3	12	31	32	33	63	80	203	204	205	235	377
Alpha	L							K	R		F	
Beta										I		
Gamma							R	K	R			
Delta						G		M				Y
Omicron		L	DEL	DEL	DEL			K	R			

Deletion 106-108: antagonism with host response to type I interferon

P323L: Alteration in the proofreading activity with increasing in mutation rate

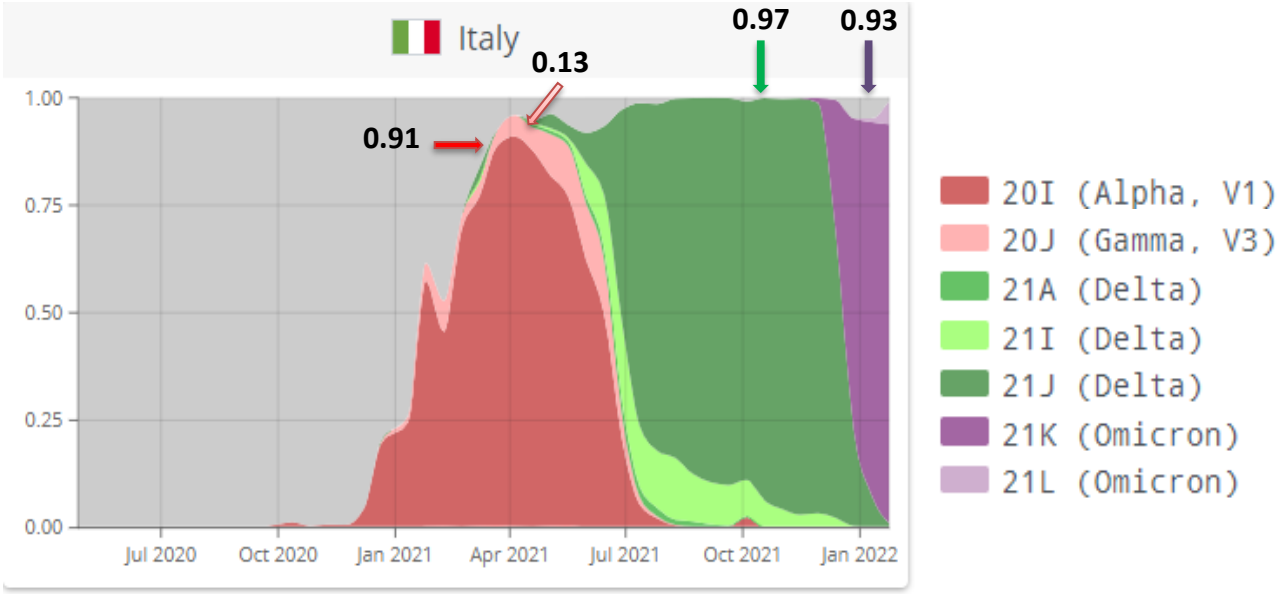
D3L mutation: reduced susceptibility (>112 fold) to several type I interferons inserting a TRS (transcription regulatory sequence) to overexpress an interferon antagonist gene

R203K/M and G204R: increased subgenomic RNA expression

https://covariants.org/variants/

VOC transmissibility in Italy

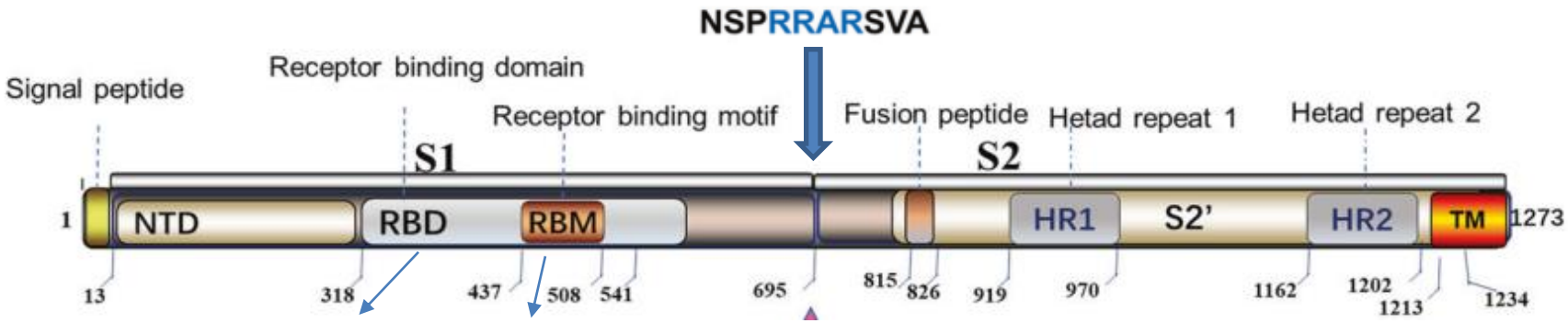
Proportion of SARS-CoV-2 variants over time



https://covariants.org/per-country

Note: The Beta variant did not become prevalent in Italy

Mutations involved in escape from immunity following natural infection and vaccination



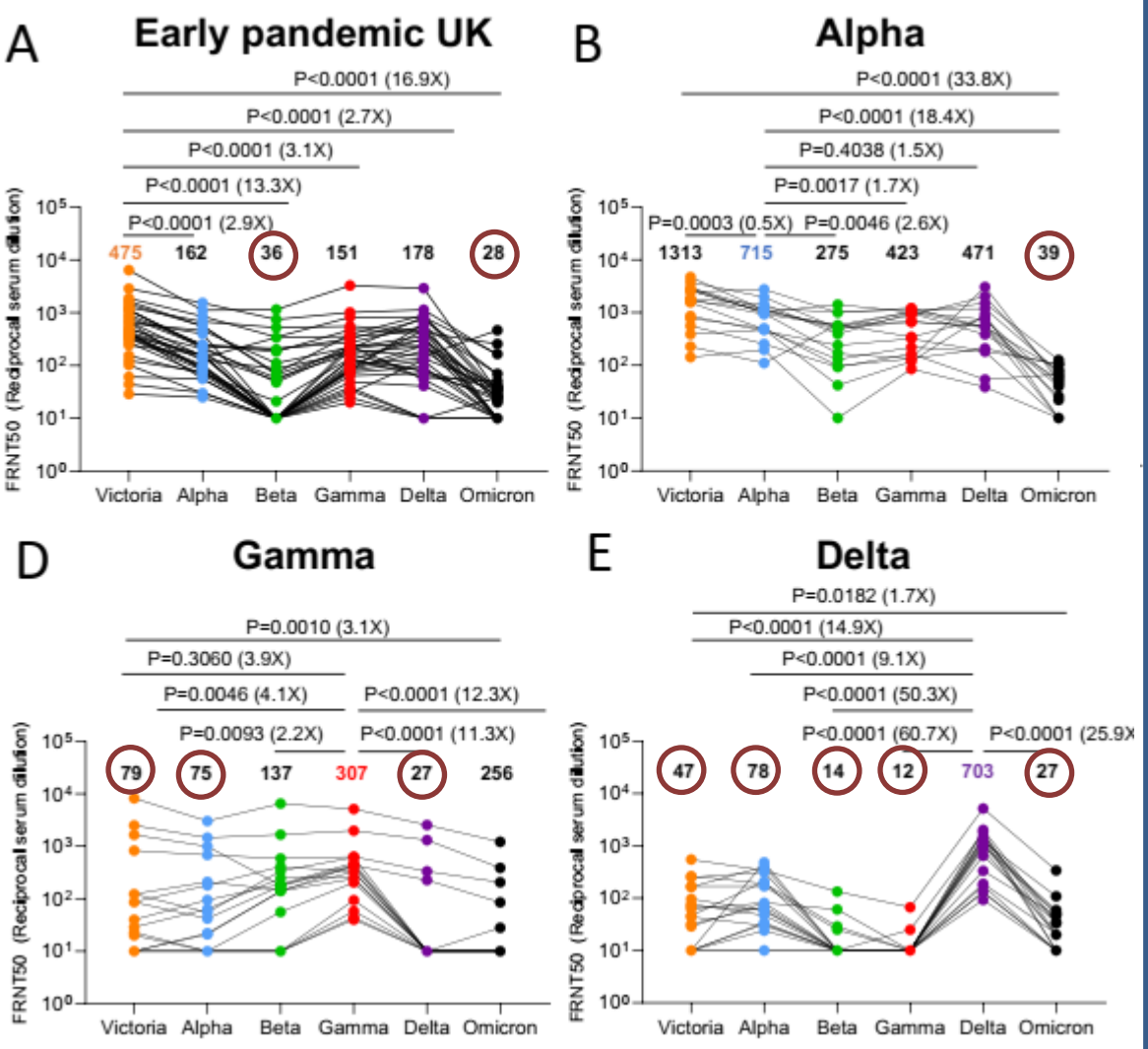
S371L and S375F (Omicron); K417N/T (Beta, Gamma, Omicron)

N440K (Omicron); L452R/Q (Delta, no vaccine escape only convalescent sera); S477N (Omicron); E484K (Beta, Gamma); G496S and Q498R (Omicron), N501Y (Alpha, Beta, Gamma, Omicron)

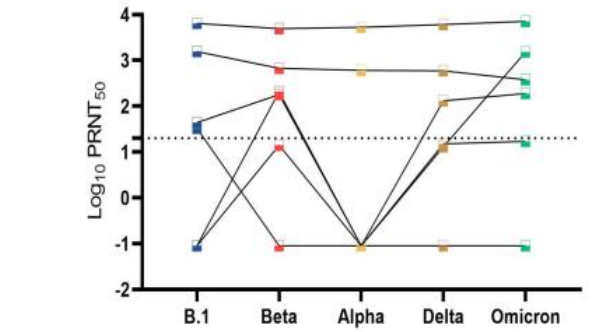
- Mutations occur in the Receptor Binding Domain (RBD) or in the Receptor binding motif (RBM), the epitopes targeted by most potent neutralizing antibodies
- For Omicron variant, changes at residues 498 and 501 of the RBD strongly impaired antibody neutralization

- Technical caveat: different methods for measuring neutralizing antibody may not correlate well

Neutralization in convalescent people

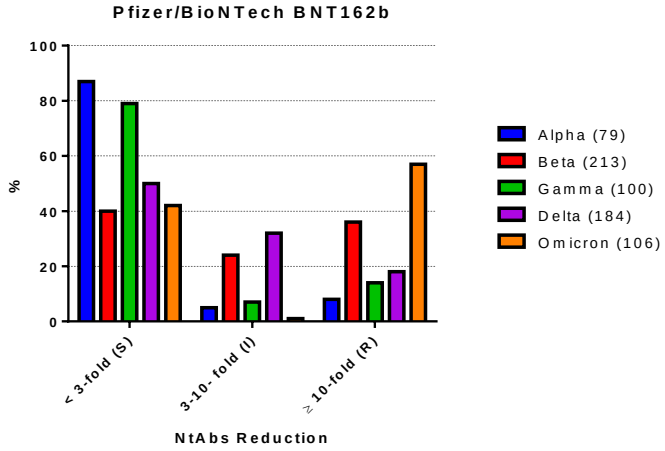


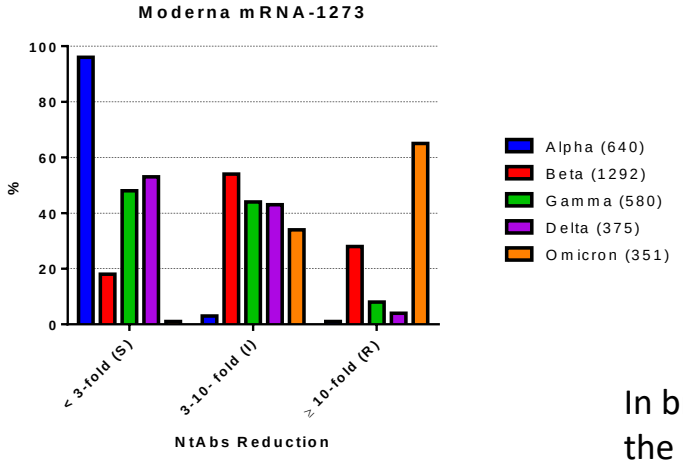
FRNT50 values for the indicated viruses using serum from convalescent subjects previously infected with A) Early pandemic virus (n=32), (B) Alpha (n=18), (C) Beta (n=14), (D) Gamma (n=16), (E) Delta (n=19)
https://doi.org/10.1101/2021.12.03.471045 preprint



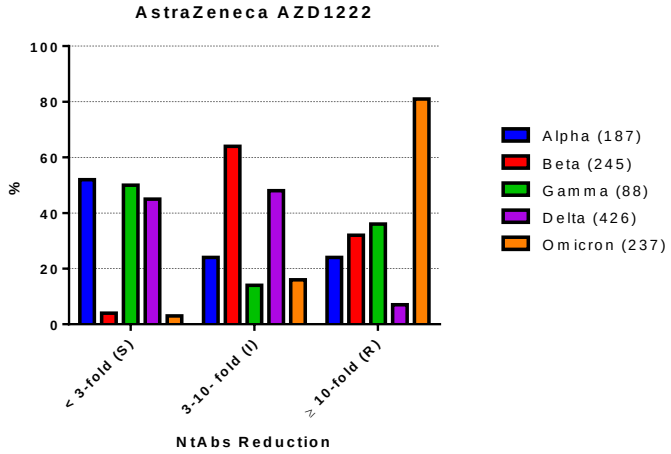
doi: https://doi.org/10.1101/2022.01.24.477043

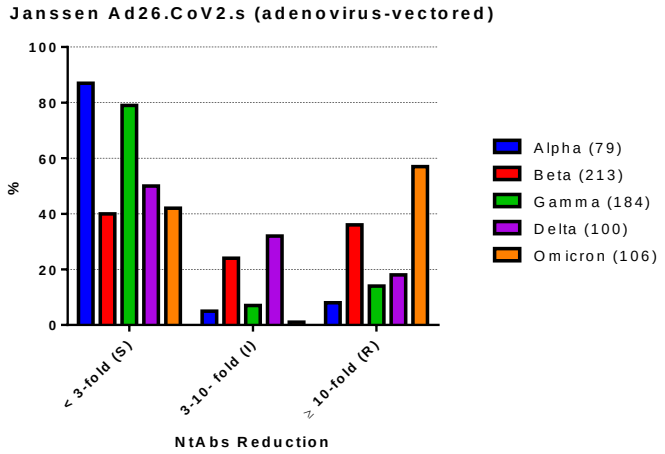
Vaccine efficacy against different variants





In bracket is indicated the number of studies





- Data were extrapolated from Stanford database (https://covdb.stanford.edu/page/susceptibility-data/) containing more than 18,000 sera tested in different studies
- 70% NtAbs titers were obtained from persons receiving an authorized mRNA vaccine (Pfizer(46%) and Moderna (22%)), 7% from recipients of the adenovirus-vectored AstraZeneca vaccine and 4% from recipients of the adenovirus-vectored Janssen vaccine

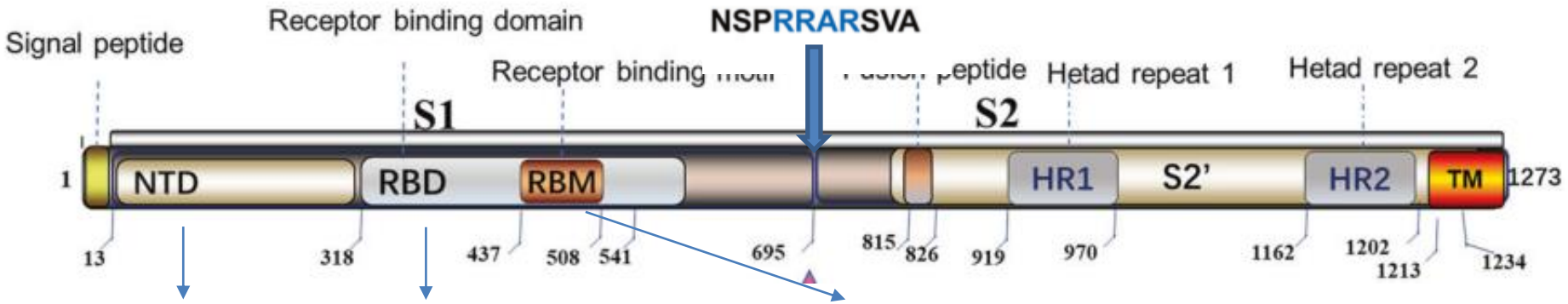
Neutralizing Monoclonal Antibodies (mAbs) in clinical trials

Name	Synonyms	Company	PDB Code	Epitope – Class[3]	NCT Trial ID
Bamlanivimab	LY-CoV555	Eli Lilly	7KMG	RBM-2	NCT04411628;NCT04427501;NCT04497987
Etesevimab	CB6; JS016; LY-CoV16	Eli Lilly	7C01	RBM-1	NCT04441918;NCT04441931;NCT04427501
Casirivimab	REGN10933	Regeneron	6XDG	RBM-1	NCT04425629;NCT04426695;NCT04452318
Imdevimab	REGN10987	Regeneron	6XDG	RBD-Core-1	NCT04425629;NCT04426695;NCT04452318
Cilgavimab	COV2-2130; AZD1061	AstraZeneca	7L7E	RBM-2	NCT04507256;NCT04625725;NCT04625972
Tixagevimab	COV2-2196; AZD8895	AstraZeneca	7L7D	RBM-1	NCT04507256;NCT04625725;NCT04625972
Sotrovimab	S309; VIR-7831; GSK4182136	Vir biotechnology	6WPS	RBD-Core-1	NCT04545060
Vir-7832		Vir biotechnology		RBD-Core-1	NCT04746183
Regdanvimab	CT-P59	Celltrion	7CM4	RBM-1	NCT04525079;NCT04593641;NCT04602000
C135		Bristol-Myers	7K8Z	RBD-Core-1	NCT04700163
C144		Bristol-Myers	7K90	RBM-3	NCT04700163
ADG20		Adagio Therapeutics		RBM	NCT04805671;NCT04859517
BRII-196	P2C-1F11	Brii Biosciences	7CDI	RBM-1	NCT04479631
BRII-198	P2B-1G5	Brii Biosciences			NCT04479644

The following mAbs are being studied in combination – Casirivimab/Imdevimab, Cilgavimab/Tixagevimab, C135/C144, and BRII-196/BRII-198. The FDA has granted emergency use authorization (EUA) for the use Sotrovimab, bamlanivimab/etesevimab, and casirivimab/imdevimab.

Tao et al., Nature Reviews 2021

Mutations conferring escape from mAbs



L18F, T20N (Gamma); D80A/G (Beta); **Del** H69, V70, V143, Y144, Y145 (Alpha and Omicron); G142D (Omicron, Delta); D215G (Beta) **Del** L242, A243, L244 (Beta)

G339D, S371L, S373P, S375F (Omicron); K417N/T (Beta, Gamma, Omicron)

N440K, G446S (Omicron), L452R (Delta), S477N (Omicron); T478K (Delta, Omicron), E484A/K/Q, N501Y (Alpha, Beta, Gamma, Omicron); Y505H

Mutations are located in epitopes targeted by different monoclonal antibodies: reduction or complete loss of neutralization efficacy

Adapted by Alkhatib et al., 2021 Microbiology Spectrum

SARs-CoV-2 VOCs and their fold reduction in neutralization susceptibility to monoclonal antibodies in advanced clinical trials

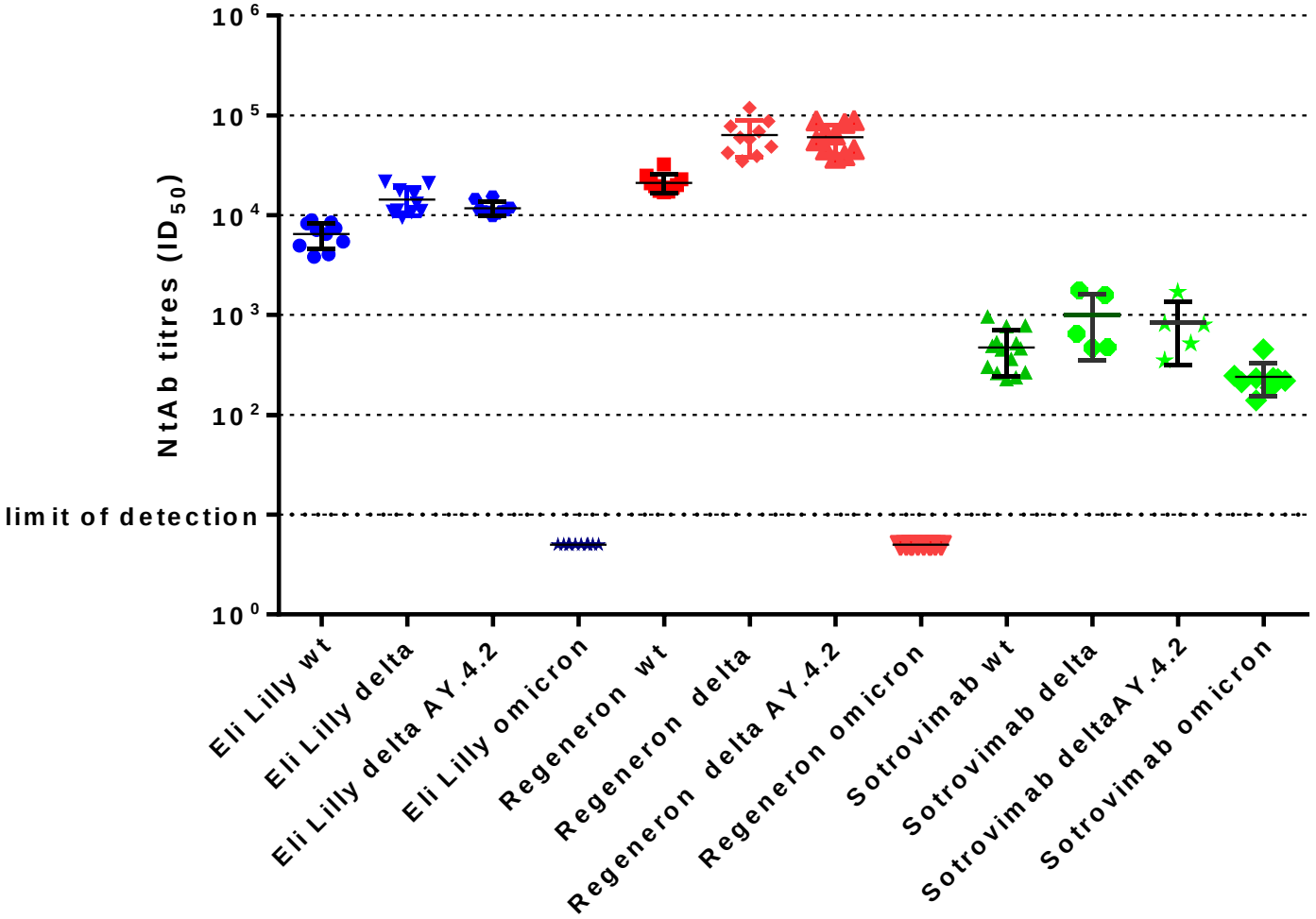
Fold reductions in neutralization (relative to control virus) for pseudo-typed and infectious viruses against the 5 VOCs (Alpha, Beta, Gamma, Delta, Omicron). Fold change is the median value of results, subscript is the number of results.

VOC	BAM	ETE	BAM/ETE	CAS	IMD	CAS/IMD	CIL	TIX	CIL/TIX	SOT	REG
Alpha	1 [17]	11 [15]	1.3 [7]	1 [23]	0.7 [23]	1 [11]	0.7 [10]	1.5 [9]	0.8 [7]	2.2* [18]	2.6 [2]
Beta	>1000 [19]	382 [17]	897 [8]	106 [27]	0.6 [26]	1.5 [14]	1.1 [9]	6.3 [9]	1.3 [7]	1 [17]	33 [3]
Gamma	>1000 [12]	304 [12]	227 [2]	177 [18]	0.3 [17]	1 [6]	0.5 [9]	6.4 [8]	0.7 [4]	1.2 [13]	61 [3]
Delta	>1000 [17]	0.4 [17]	0.9 [6]	0.7 [18]	1.5 [18]	1 [7]	2.5 [6]	0.6 [6]	0.7 [4]	1.3 [11]	9.8 [3]
Omicron	>1000 [20]	323 [19]	897 [8]	>1000 [21]	>1000 [21]	>1000 [10]	305 [18]	276 [18]	59 [10]	3.4 [20]	>1000 [7]

BAM, bamlanivimab (LY-CoV555); CAS, casirivimab (REGN10933); CIL, cilgavimab (AZD1061); ETE, etesevimab (LY-CoV016); IMD, imdevimab (REGN10987); REG, regdanvimab (CT-P59); SOT, sotrovimab (Vir-7831); TIX, Tixagevimab (AZD8895)

https://covdb.stanford.edu/page/susceptibility-data/

Neutralization of mAbs against different variant



El Lilly: Etesevimab+Bamlanivimab; Regeneron: Casirivimab+Imdevimab; delta AY 4.2: delta plus additional mutation Y145H and A222V; ID50: Drug Concentration neutralizing the 50% of virus in a microneutralization assay in VERO E6 using live virus

Dragoni et al., submitted to CMI 2022

Impact of SARS-CoV-2 variability on diagnostics

- Most assays target multiple genome regions to minimize false negatives
- Failure to detect specific targets can be used for presumptive variant assignment:
  - S-gene dropout: spike amplification failure with del 69-70 in alpha and omicron (BA.2)
- Diagnostic targets should be reviewed on a regular basis by manufacturers and by laboratories
  - Delay between identification of a new variant and availability of updated kit

Companies rarely or never disclose detailed Information on their assays

Selection of available PCR assays and the impacts on their performance of the Alpha and Omicron variants

		Targets						Reported impact of VOC on signal detection of the S-gene target	
Supplier in Switzerland and Liechtenstein	**Test-kit**	**ORF1ab**	**RdRp**	**S**	**E**	**M**	**N**	**Alpha (B.1.1.7)**	**Omicron (B.1.1.529)**
Abbott	Abbott RealTime SARS-CoV-2 (#09N77-095)	0	1	0	0	0	1	No S-gene target	No S-gene target
Abbott	ALINITY m SARS-COV-2 ASSAY (#09N78-095)	0	1	0	0	0	1	No S-gene target	No S-gene target
Altona	Altona "RealStar® SARS-CoV-2 RT-PCR" (#821005)	0	0	1	1	0	0	No impact	No impact
Axon Lab / Cepheid	Gene Xpert® Xpress SARS-CoV-2 (#12039255)	0	0	0	1	0	1	No S-gene target	No S-gene target
Axon Lab	BioGX SARS-CoV-2 kit (#444213)	0	0	0	0	0	2	No S-gene target	No S-gene target
Axon Lab	EASY® SARS-CoV-2 Kit (#RT020)	0	1	0	0	0	1	No S-gene target	No S-gene target
Axon Lab	Hi-PCR® Coronavirus (SARS-CoV-2) Probe PCR Kit (#MBPCR242)	0	1	0	0	0	0	No S-gene target	No S-gene target
Axon Lab	NaGene Multiple Real-Time PCR kit for Detection of 2019-nCoV	1	0	0	0	0	1	No S-gene target	No S-gene target
Axon Lab	Progenie Coronavirus 2019-nCoV Assay (#CORO-UX 5.2)	0	0	0	0	0	1	No S-gene target	No S-gene target
Becton Dickinson	ViaSure SARS-CoV-2 (N1+N2) Real Time PCR (#444215)	0	0	0	0	0	2	No S-gene target	No S-gene target
Becton Dickinson	ViaSure SARS-CoV-2 S gene Real Time PCR (#444212)*	0	0	1	0	0	0	Detection compromised*	Not available in EU/CHE*
Becton Dickinson	ViaSure SARS-CoV-2, Flu (A+B) & RSV Real Time PCR Detection Kit (#444217)	0	0	0	0	0	2	No S-gene target	No S-gene target
bioMerieux	BIOFIRE® COVID-19 test (#423744)	1	0	0	0	0	0	No S-gene target	No S-gene target
bioMerieux	BIOFIRE® FILMARRAY® Respiratory Panel 2.1+ (RP2.1+) (#423740)	0	0	1	0	1	0	No impact	No impact
bioMerieux	SARS-COV-2 R-GENE® (#423720)	0	1	0	1	0	1	No S-gene target	No S-gene target
Bühlmann	Seegene Allplex™ 2019-nCov (#RP10243X) (older Kit)	0	1	0	1	0	1	No S-gene target	No S-gene target
Bühlmann	Seegene Allplex™ SARS-CoV-2 (#RV10248X) (newer kit)	0	1	1	1	0	1	2x 1-mer mismatch**	No impact
Bühlmann	Seegene Allplex™ SARS-CoV-2/Flu A/Flu B/RSV Assay (#RV10259X)	0	1	1	0	0	1	1x 1-mer mismatch**	Mismatch (delayed Ct/drop out possible)
DiaSorin	Simplexa™ COVID-19 Direct (#MOL4150)	1	0	1	0	0	0	No impact	No impact
Ender diagnostics	Ender MASS	1	0	0	0	0	0	No S-gene target	No S-gene target
Hologic	Panther® Fusion SARS-CoV-2 (#AW-21159-001)	2	0	0	0	0	0	No S-gene target	No S-gene target
Hologic	Aptima® SARS-CoV-2 Assay (#PRD-06419)	2	0	0	0	0	0	No S-gene target	No S-gene target
Hyris	SARS-CoV-2 human diagnostics (#bKTH-SCV2.02)	0	0	0	0	0	2	No S-gene target	No S-gene target
FRIZ Biochem	COVID-19 direct RT-PCR (#FBC101)	0	0	0	1	0	1	No S-gene target	No S-gene target
Lubioscience	2019-nCoV CDC EUA Kit (#10006606)	0	0	0	0	0	2	No S-gene target	No S-gene target
Mikrogen Diagnostik	ampliCube Coronavirus SARS-CoV-2 (#50143, #50144)	1	0	0	1	0	0	No S-gene target	No S-gene target
Qiagen	QiaStat-Dx Respiratory SARS-CoV-2 Panel (#691223)	0	1	0	1	0	0	No S-gene target	No S-gene target
Qiagen	NeuMoDx SARS-CoV-2 Assay (#300800)	1	0	0	0	0	1	No S-gene target	No S-gene target
Qiagen	SARS-CoV-2 N1+N2 Assay Kit (#222015 or #222017)	0	0	0	0	0	2	No S-gene target	No S-gene target
Roche Diagnostics	cobas® 6800/8800 SARS-CoV-2 (#09175431190)	1	0	0	1	0	0	No S-gene target	No S-gene target
Roche Diagnostics	cobas® 6800/8800 SARS-CoV-2 & Influenza A/B (#09233474190)	1	0	0	1	0	0	No S-gene target	No S-gene target
Roche Diagnostics	cobas® Liat SARS-CoV-2 & Influenza A/B (#09211101190)	1	0	0	0	0	1	No S-gene target	No S-gene target
Sansure	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (#S3104E)	1	0	0	0	0	1	No S-gene target	No S-gene target
Siemens Healthineers	FTD SARS-CoV-2 qPCR Test (#11416302)	1	0	0	0	0	1	No S-gene target	No S-gene target
TECOmedical	PathoFinder RealAccurate® Quadruplex SARS-CoV-2 PCR Kit (#PF0971C-R)	0	1	0	0	0	1	No S-gene target	No S-gene target
TECOmedical	Eurobio Scientific EurobioPlex SARS-CoV-2 Multiplex (#EBX-041-192)	0	2	0	0	0	1	No S-gene target	No S-gene target
Thermo Fisher	TaqPath™ COVID-19 Combo Kit (#A48067)	1	0	1	0	0	1	S-gene drop out	S-gene drop out
Thermo Fisher	TaqPath™ COVID-19, FluA/B, RSV (#A49867)	0	0	1	0	0	1	No impact	No impact
TibMolBio	LightMix® Modular Sarbecovirus SARS-CoV-2 (#50-0776-96)	0	1	0	1	0	1	No S-gene target	No S-gene target

Conclusions

- Limited evolutionary rate, however, variants emerge in the pandemic context
  - Recombination across host species, jumping back to humans
- Escape from immunity following natural infection is a concern
  - Waning immunity is an intrinsic feature of coronaviruses
- Escape from vaccine elicited immunity likely due to outdated immunogen
  - Updating in progress but may run out of time
- Escape from diagnostics is limited
  - Multitarget strategy
- Unprecedented sequencing of a massive number of isolates worldwide
  - Far from being uniform worldwide

Thank you for your attention