Discovery and optimization of neutralizing SARS-CoV-2 antibodies using ALTHEA Gold Plus Libraries™

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Abstract

We recently reported the isolation and characterization of anti-SARS-CoV-2 antibodies from a phage display library built with the VH repertoire of a convalescent COVID-19 patient, paired with four naïve synthetic VL libraries. One of the antibodies, called IgG-A7, neutralized the Wuhan, Delta (B.1.617.2) and Omicron (B.1.1.529) strains in authentic neutralization tests (PRNT). It also protected 100% transgenic mice expressing the human angiotensin-converting enzyme 2 (hACE-2) from SARSCoV-2 infection. In this study, the four synthetic VL libraries were combined with the semi-synthetic VH repertoire of ALTHEA Gold Libraries™ to generate a set of fully naïve, general-purpose, libraries called ALTHEA Gold Plus Libraries™. Three out of 24 specific clones for the RBD isolated from the libraries, with affinity in the low nanomolar range and sub-optimal in vitro neutralization in PRNT, were affinity optimized via a method called "Rapid Affinity Maturation" (RAM). The final molecules reached sub-nanomolar neutralization potency, slightly superior to IgG-A7, while the developability profile over the parental molecules was improved. These results demonstrate that general-purpose libraries are a valuable source of potent neutralizing antibodies. Importantly, since general-purpose libraries are "ready-to-use", it could expedite isolation of antibodies for rapidly evolving viruses such as SARS-CoV-2.

1 ALTHEA Gold Plus Libraries™ Construction and Quality Control

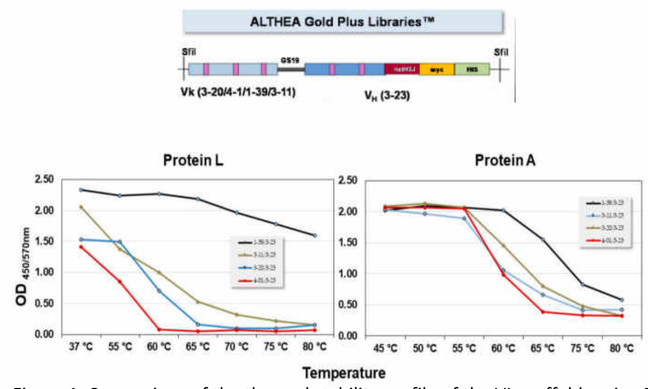


Figure 1. Comparison of the thermal stability profile of the VL scaffolds using Protein L and Protein A

The selection of thermostable scFvs, was based on the natural capacity of the Protein L of *Peptostreptococcus magnus* to bind the framework 1 of the synthetic VL libraries, after a heat shock of 10 min at **55 °C.**

2 Selection of Anti-SARS-CoV-2 Neutralizing Antibodies

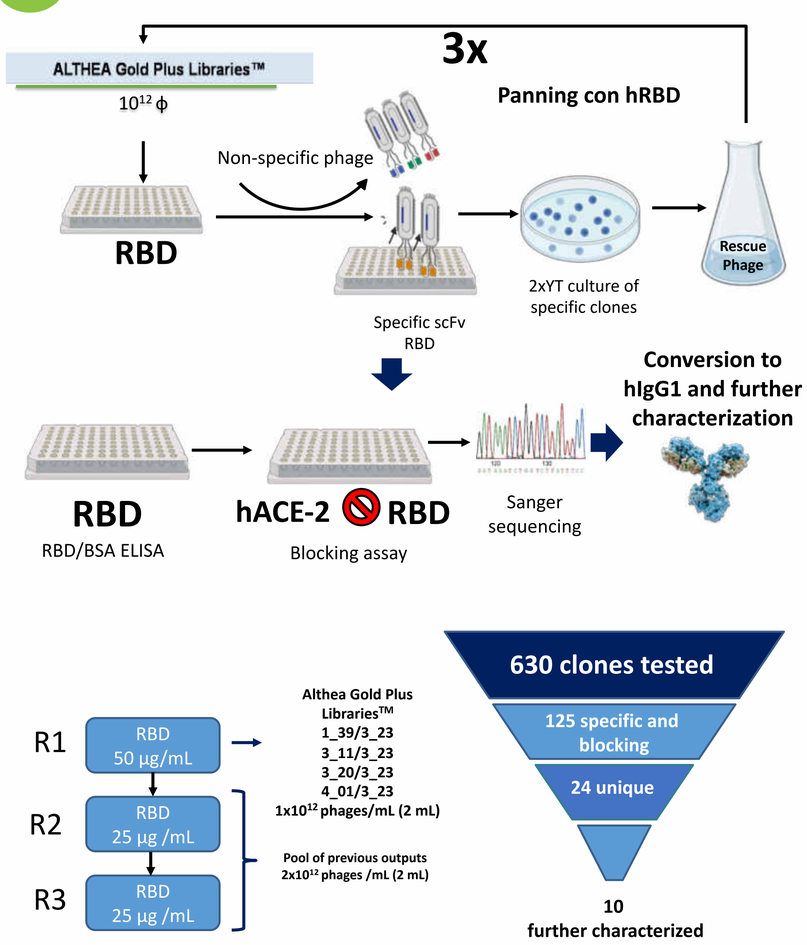


Figure 2. 630 clones were tested for binding to RBD, yielding 24 positive and unique clones. Ten were converted to hIgG1, expressed in HEK293 cells and further characterized. Two of the clones, called **P5E1 and P5A10** neutralized the viral entry into Vero cells and were selected for affinity maturation.

Table 1. Summary of characterization of P5E1 and P5A10 antibodies

Assay		Units	P5E1	P5A10	IgG-A7 (1)	CB6
Monomeric Content		%/kDa	95.6/161	96.8/177	100/138.1	94.7/146.0
Thermal Stability	Tm1	°C	64.3	69.3	68.5	69.3
	Tm2	°C	83.6	82.0	82.1	82.0
ELISA	EC_{50}	nM	0.97	16.87	0.025	0.027
SPR	ka	1/Ms	1.60×10^5	1.27×10^5	5.20×10^5	1.10×10^6
	kd	1/s	4.33×10^{-3}	3.67×10^{-3}	3.55×10^{-4}	1.32×10^{-2}
	K_D	nM	27.09	28.78	0.68	12.06
Competition	IC_{50}	nM	2.15	2.34	0.19	0.49
Blocking	IC_{50}	nM	2.63	–	0.25	0.30–1.29
Neutralization	NC_{50}	nM	80% neutralization at 100 µg/mL	80% neutralization at 100 µg/mL	0.56	0.56–2.74 (2)

(1) Data reported in [2,3]. (2) A range of values is reported to capture the variability of several neutralization assays reported here (see figure 6) and those reported in a previous publication [2]. CB6 (precursor of etesevimab) as reference antibody.

3 P5E1 and P5A10 VH RAM (Rapid Affinity Maturation)

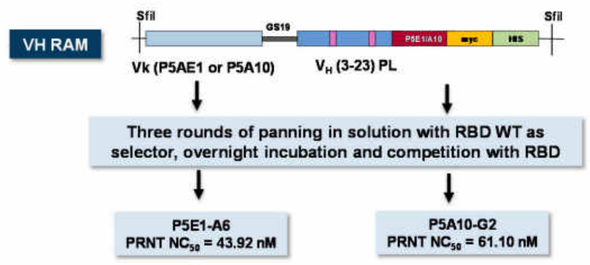


Figure 3. Starting from P5E1 and P5A10, a second set of antibodies was generated by reshuffling diversity in the HCDR1 and HCDR2. The clones with best ELISA binding to RBD were converted to hIgG1, expressed in HEK 293 cells and further characterized yielding two final candidates P5E1-A6, P5A10-G2 and PA10-G4

Table 2. Summary of characterization of P5E1-A6 and P5A10-G2 and P5A10-G4

Assay		Units	P5E1-A6	P5A10-G2	P5A10-G4
Monomeric Content		%/kDa	100/151.0	100.0/165.0	99.1/151.0
Thermal Stability	Tm1	°C	68.2	68.5	64.3
	Tm2	°C	78.2	81.1	76.3
SPR	ka	1/Ms	5.88×10^5	1.49×10^6	1.09×10^6
	kd	1/s	5.27×10^{-4}	5.78×10^{-4}	1.48×10^{-4}
	K_D	nM	0.89	0.39	0.14
Competition	IC_{50}	nM	0.06	1.23	0.12
Blocking	IC_{50}	nM	0.61	1.18	0.78
Neutralization	NC_{50}	nM	43.92	61.10	10.45

Epitope Mapping and Mechanism of Neutralization of P5E1-A6, P5A10-G2 and IgG-A7

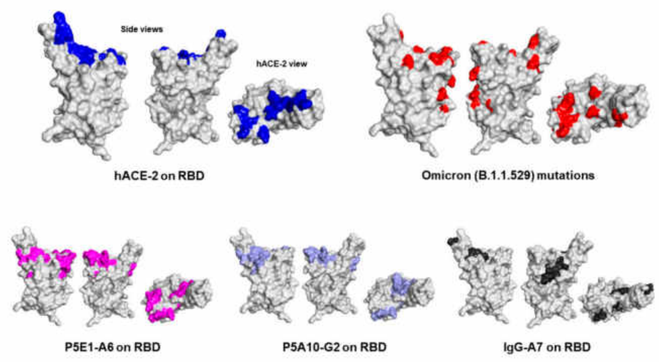


Figure 4. Epitope of P5E1-A6 (Violet), P5A10-G2 (light blue) and IgG-A7 (Black) on the Connolly surface of SARS-CoV-2 RBD WT. As a reference, we show, on the top left, two side views of the RBD rotated 180◦ and the top view of RBD interface (dark blue) with the hACE-2 (hACE-2 view). On the top right, Omicron mutations (red) with respect to Wuhan RBD. The figures were prepared with the PDB ID: 7SWP in Discovery Studio 2020 v20.1.0.19295 (BIOVIA)

4 P5E1-A6 VL RAM

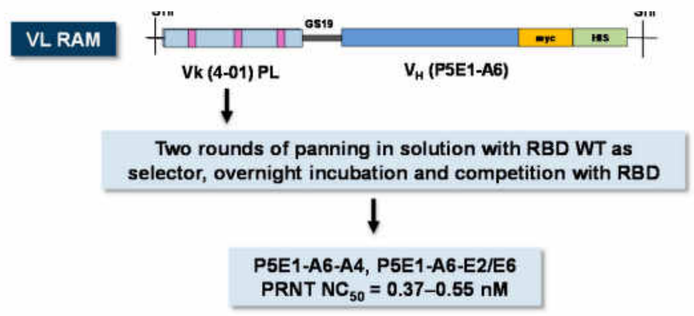
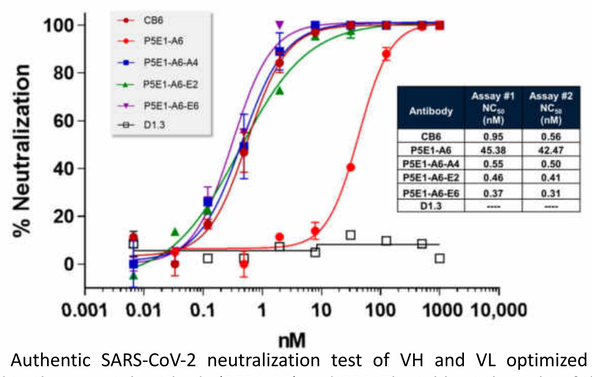


Figure 5. Starting from P5E1-A6, a third set of antibodies was generated by reshuffling diversity at VL by combining the VH sequence of P5E1-A6 with the synthetic library corresponding to its VL (4-01). The clones with best ELISA binding to RBD were converted to hIgG1, expressed in HEK293 cells and further characterized yielding four final candidates **P5E1-A6-A4, P5E1-A6-E2 & P5E1-A6-E6**

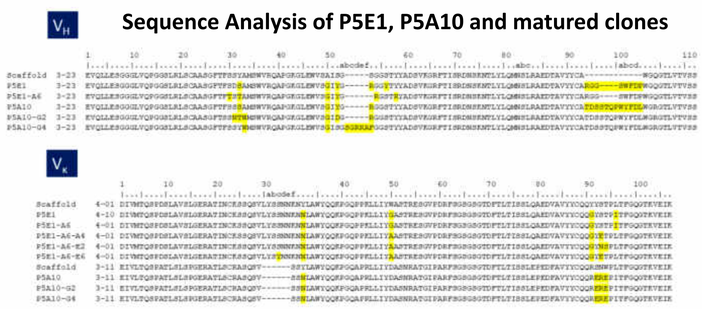
Plaque Reduction Neutralization Test (PRNT)



Antibody	Assay #1 NC_{50} (nM)	Assay #2 NC_{50} (nM)
CB6	0.95	0.56
P5E1-A6	45.38	42.47
P5E1-A6-A4	0.55	0.50
P5E1-A6-E2	0.46	0.41
P5E1-A6-E6	0.37	0.31
D1.3	----	----

Figure 6. Authentic SARS-CoV-2 neutralization test of VH and VL optimized antibodies as compared to the parental antibody (P5E1-A6) and CB6. The table at the right of the plot reports the value of the neutralization potency of two assays performed in different days. The plot corresponds with Assay #1 dataset

V_H Sequence Analysis of P5E1, P5A10 and matured clones



V_K

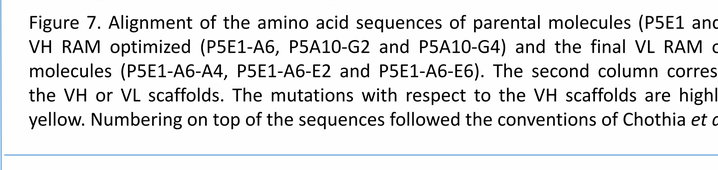


Figure 7. Alignment of the amino acid sequences of parental molecules (P5E1 and P5A10), VH RAM optimized (P5E1-A6, P5A10-G2 and P5A10-G4) and the final VL RAM optimized molecules (P5E1-A6-A4, P5E1-A6-E2 and P5E1-A6-E6). The second column corresponds to the VH or VL scaffolds. The mutations with respect to the VH scaffolds are highlighted in yellow. Numbering on top of the sequences followed the conventions of Chothia *et al*.

5 Comparison of the outcome of the selections from ALTHEA Gold Plus Libraries™ with the immune library

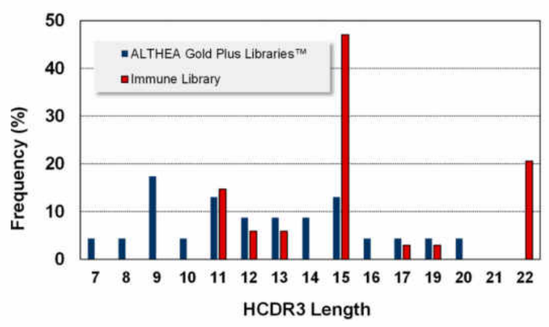
Table 3. VL scaffold usage

Scaffold	ALTHEA Gold Plus Libraries™	Immune Library
1-39	38%	35%
3-11	21%	6%
3-20	13%	47%
4-01	13%	9%

The 1-39 scaffold was the best one performing in the filtration process to generate ALTHEA Gold Plus libraries™ (see Figure 1). This might explain the higher frequency of this scaffold in both libraries.

Figure 8. HCDR3 length frequency of the selected clones from ALTHEA Gold Plus libraries™ and the immune library.

Notably, similar to VL, the HCDR3 of the antibodies isolated from ALTHEA Gold Plus libraries™ were more evenly distributed than those of the immune libraries.



Summary

- A panel of anti-SARS CoV-2 antibodies was isolated from ALTHEA Gold Plus Libraries™ using RBD as selector. 630 clones were tested for binding to RBD, yielding 24 positive and unique clones.
- A second set of antibodies was generated by reshuffling diversity in the HCDR1 and HCDR2. The lead molecule P5E1-A6 has a developability profile consistent with a therapeutic antibody.
- A third set de antibodies increased the affinity and neutralization potency (P5E1-A6-A4, P5E1-A6-E2 & P5E1-A6-E6).
- We have demonstrated that naïve general-purpose synthetic VH repertoires produce similar results than those obtained from an immune VH repertoire.

References

[1]. Guzmán-Bringas et al., Discovery and Optimization of Neutralizing SARS-CoV-2 Antibodies Using ALTHEA Gold Plus Libraries™. *Int. J. Mol. Sci.* 2023, *24*, 4609.
[2]. Mendoza-Salazar et al., Anti-SARS-CoV-2 Omicron Antibodies Isolated from a SARS-CoV-2 Delta Semi-Immune Phage Display Library. Antibodies. 2022;11:13.
[3]. Gonzalez-Gonzalez et al., In Vitro and In Vivo Characterization of a Broadly Neutralizing Anti-SARS-CoV-2 Antibody Isolated from a Semi-Immune Library. Antibodies. 2022;11:57.

Funding

This work was supported in part by a grant from AMEXID-Secretaría de Relaciones Exteriores Fondo México-Chile (CH05-Neutralizing antibodies for SARS-CoV-2 MEX-CHI). Part of the work was funded by UDIBI internal Projects: UDIP20-016, UDIP21-008 and UDIP21-014

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