

# Development of Human Antibodies Against SARS-CoV-2 Variants of Concern

Hernandez-Rivera DV<sup>1,2</sup>, Muñoz-Herrera JC<sup>1,2</sup>, Vázquez-Leyva SK<sup>1,2</sup>, Pelcastre-Gómez AY<sup>1,2</sup>, Salinas-Trujano JR<sup>1,2</sup>, Pérez-Tapia SM<sup>1,2</sup>, Pedraza-Escalona M<sup>1,2,3\*</sup>



<sup>1</sup>Unidad de Desarrollo e Investigación en Bioprocesos (UDIBI), Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, 11340, México

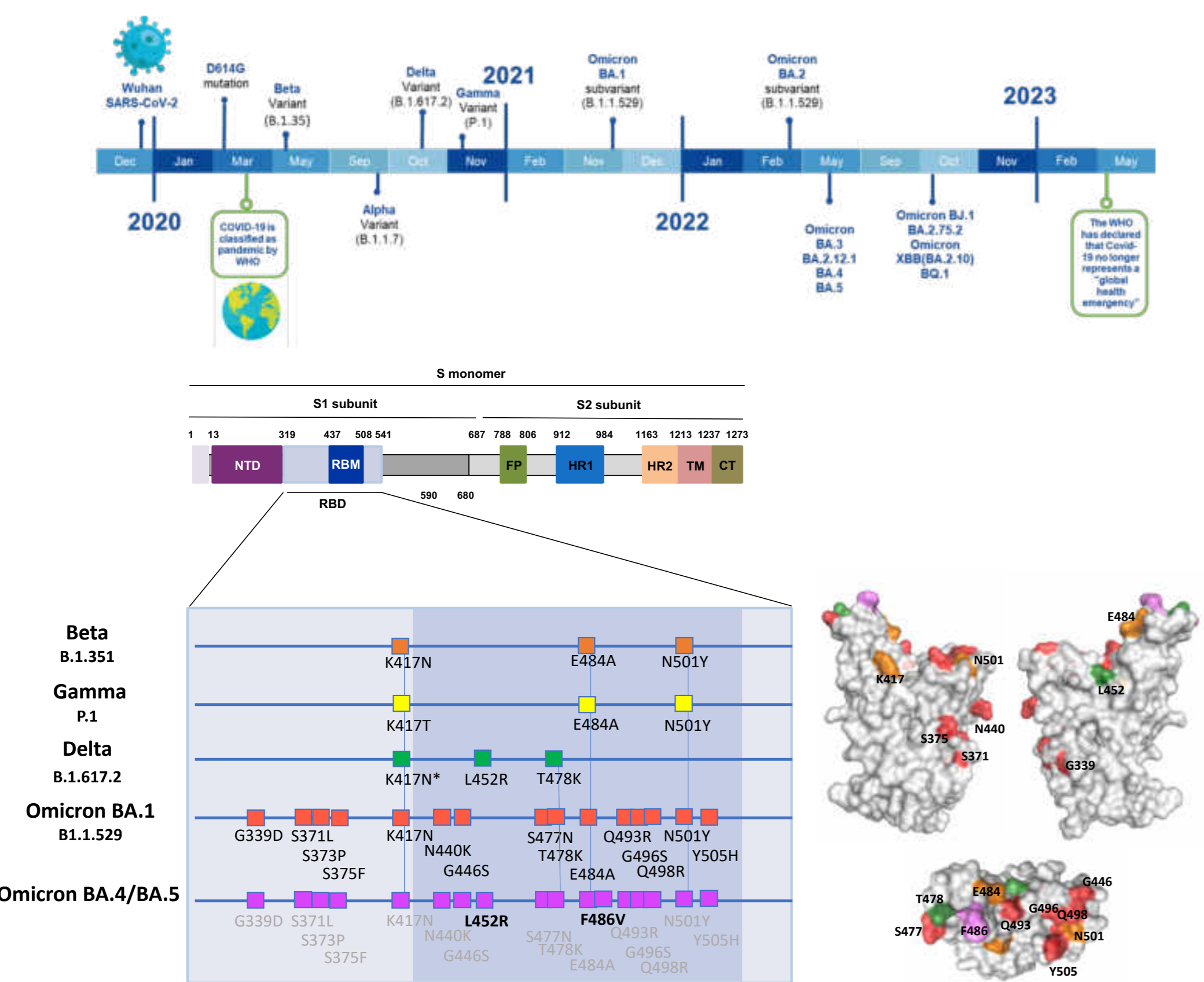
<sup>2</sup>Laboratorio Nacional para Servicios Especializados de Investigación, Desarrollo e Innovación (I+D+i) para Farmoquímicos y Biotecnológicos, LANSEIDI-FarBiotec-CONACyT, México

<sup>3</sup>CONACyT-Unidad de Desarrollo e Investigación en Bioterapéuticos (UDIBI), Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, 11340, México

\*martha.pedraza@udibi.com.mx; Tel: +525557296000 ext 62543

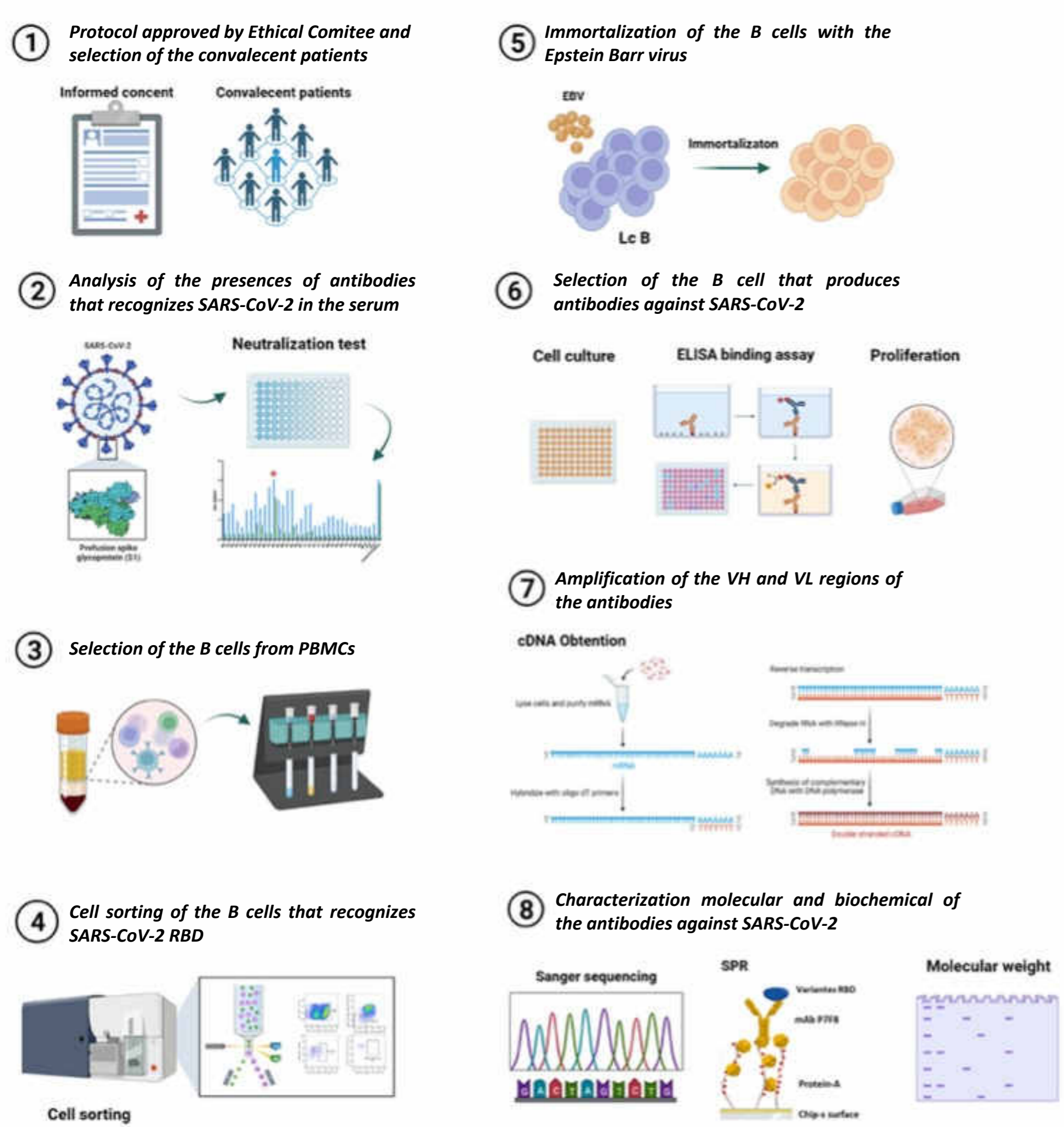
## ABSTRACT

Although the vaccination schemes against COVID-19 represented a great advance in the reduction of infection severity by increasing the immune response to patients, the high rate of mutation of SARS-CoV-2 maintains the possibility of new outbreaks. In this work, we selected and characterized a group of human monoclonal antibodies able to recognize and neutralize SARS-CoV-2 variants of concern (VOC) using the Epstein-Barr virus immortalization human B cells method. From 1.5 to 19 x10<sup>6</sup> B lymphocytes (CD22+), we obtained between 0.073% to 0.29% B cells that produce specific antibodies against RBD. After immortalization, 64 positive clones were obtained from a patient convalescent to Omicron BA.1 variant and 59 from a patient convalescent to Omicron BA.4/BA.5 variant. The lymphoblastic cell line P7F8 produces an antibody (IgG isotype) with a lambda light chain germline genes IGHV3-25\*3 and IGHV2\*01 and a heavy chain germline genes IGHV6-1\*02,IGHJ4\*02 and IGHJ3-22\*01. The last one has a YYDRxG-like motif, which has been reported to be important in the recognition and neutralization to different SARS-CoV-2 RBD variants. The antibody has been purified and the affinity constants towards different VOC have been determined by SPR: 2.056nM to RBD WT, 0.018nM to Beta variant, 0.051nM to Delta, 0.039nM to Delta plus (+K417N), 0.88 nM to Omicron BA.1 and 0.148nM to Omicron BA.4/BA.5. This antibody targets a highly conserved epitope among the different VOC, which can be used in diagnostic and therapeutic approaches.



**Figure 1. Characteristics of SARS-CoV-2 and variants of concern.** (Up) Timeline of the apparition of SARS-CoV-2 and the variants of concern. (Down) Schematic representation of the different domains in the subunit 1 and 2 of the SARS-CoV-2 spike. NTD, N-terminal domain (in purple); RBD, receptor binding domain (in light blue); RBM, receptor binding motif (in deep blue); FP, fusion peptide (in forest green); HR1, heptapeptide repeat sequence 1 (in blue); HR2, heptapeptide repeat sequence 2 (in melon orange); TM, transmembrane domain (in pink); CT and cytoplasmic tail (in gold). RBD mutations in the variants of concern and their location in the tridimensional structure. The figures were prepared using PyMOL Molecular Graphics System version 2.4.1. PDB ID: 7BNM.

## METHODS



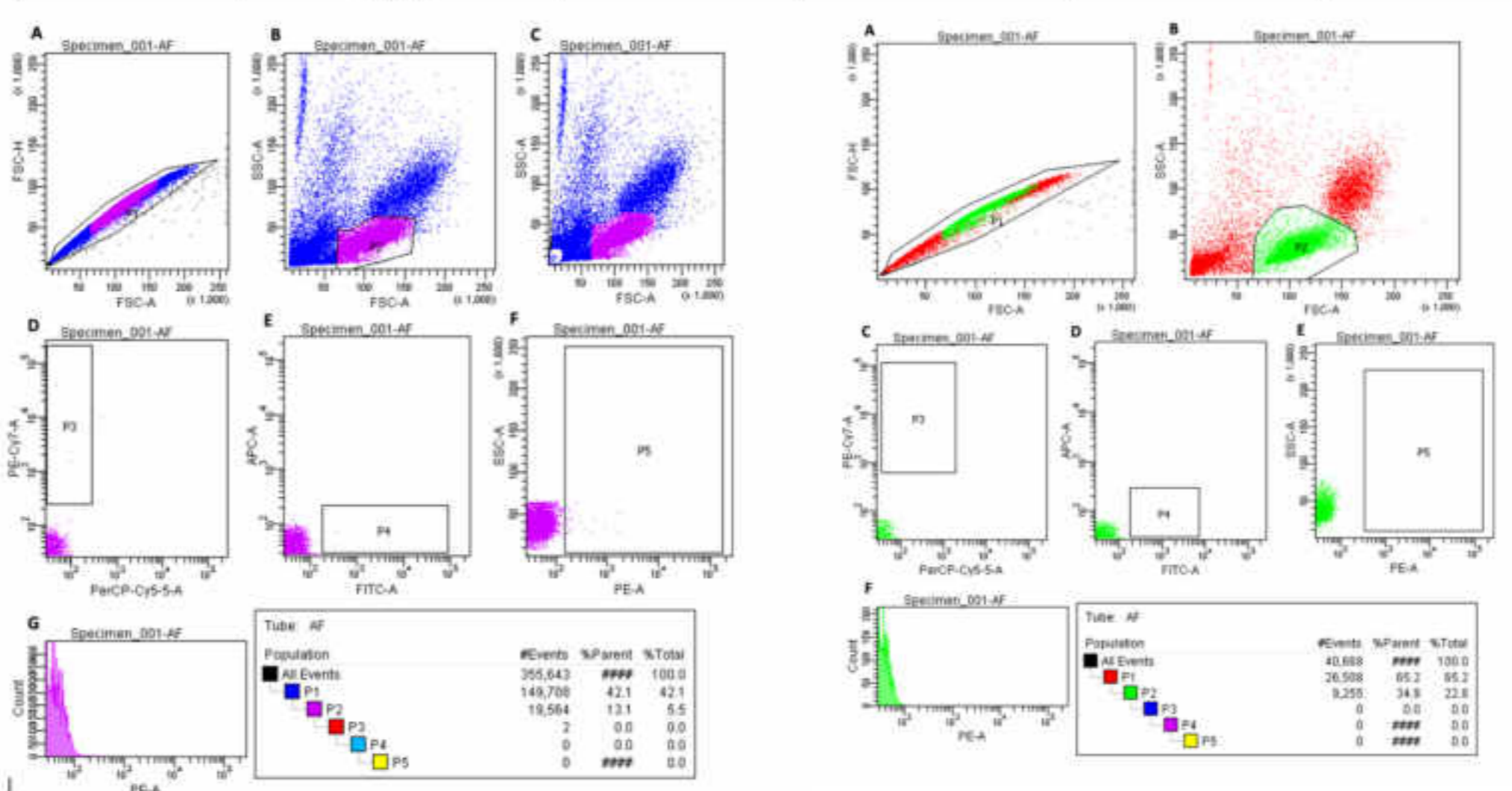
**Figure 2. Human B cell immortalization for monoclonal antibody production against SARS-CoV-2.**

## RESULTS

### Selection of the B cells that recognizes SARS-CoV-2 RBD from PBMCs

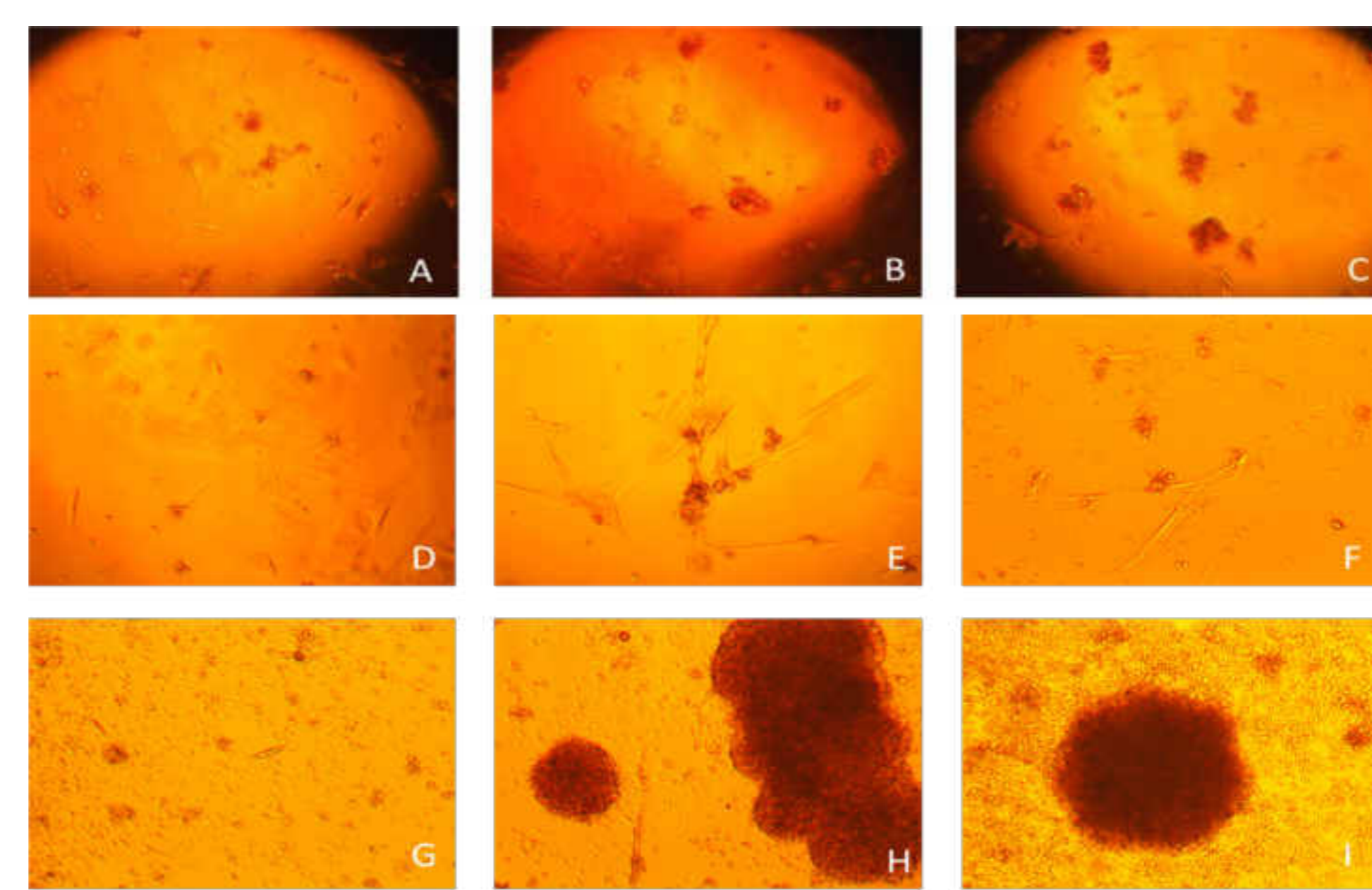
**Table 1. The frequency of SARS-CoV-2 specific-B cells in diferent patients.**

Patient code	Patient conditions	PBMCs (total quantity)	CD22 <sup>+</sup> cells	CD22 <sup>+</sup> CD20 <sup>+</sup> CD27 <sup>+</sup> CD3 <sup>+</sup> CD16 <sup>+</sup> CD14 <sup>+</sup> IgM <sup>+</sup> RBD <sup>+</sup> cells total (SARS-CoV-2 B cells sorted)	Ratio SARS-CoV-2 B cells/CD22 <sup>+</sup> cells
P1	WT convalescent/ without vaccination scheme	11.3 x 10 <sup>7</sup>	9.12 x 10 <sup>6</sup>	23278	0.25524
P2		8.8 x 10 <sup>7</sup>	15.5 x 10 <sup>6</sup>	45975	0.29661
P3		2.6 x 10 <sup>7</sup>	2.3 x 10 <sup>6</sup>	377	0.01639
P4		2.7 x 10 <sup>7</sup>	1.56 x 10 <sup>6</sup>	263	0.01685
P5		7.4 x 10 <sup>7</sup>	6.4 x 10 <sup>6</sup>	12607	0.19698
P6		7.2 x 10 <sup>7</sup>	8.07 x 10 <sup>6</sup>	12057	0.1494
P7		13 x 10 <sup>7</sup>	19 x 10 <sup>6</sup>	34772	0.18301
P8 T1	Delta convalescent/ with vaccination scheme	5.92 x 10 <sup>7</sup>	3 x 10 <sup>6</sup>	2204	0.07346
P8 T2	Omicron BA.1 convalescent/ with vaccination scheme	7.2 x 10 <sup>7</sup>	1.92 x 10 <sup>6</sup>	17967	0.9357
P9	Convalescent to several infections/ with vaccination scheme	23.6 x 10 <sup>7</sup>	7 x 10 <sup>6</sup>	5691	0.0813

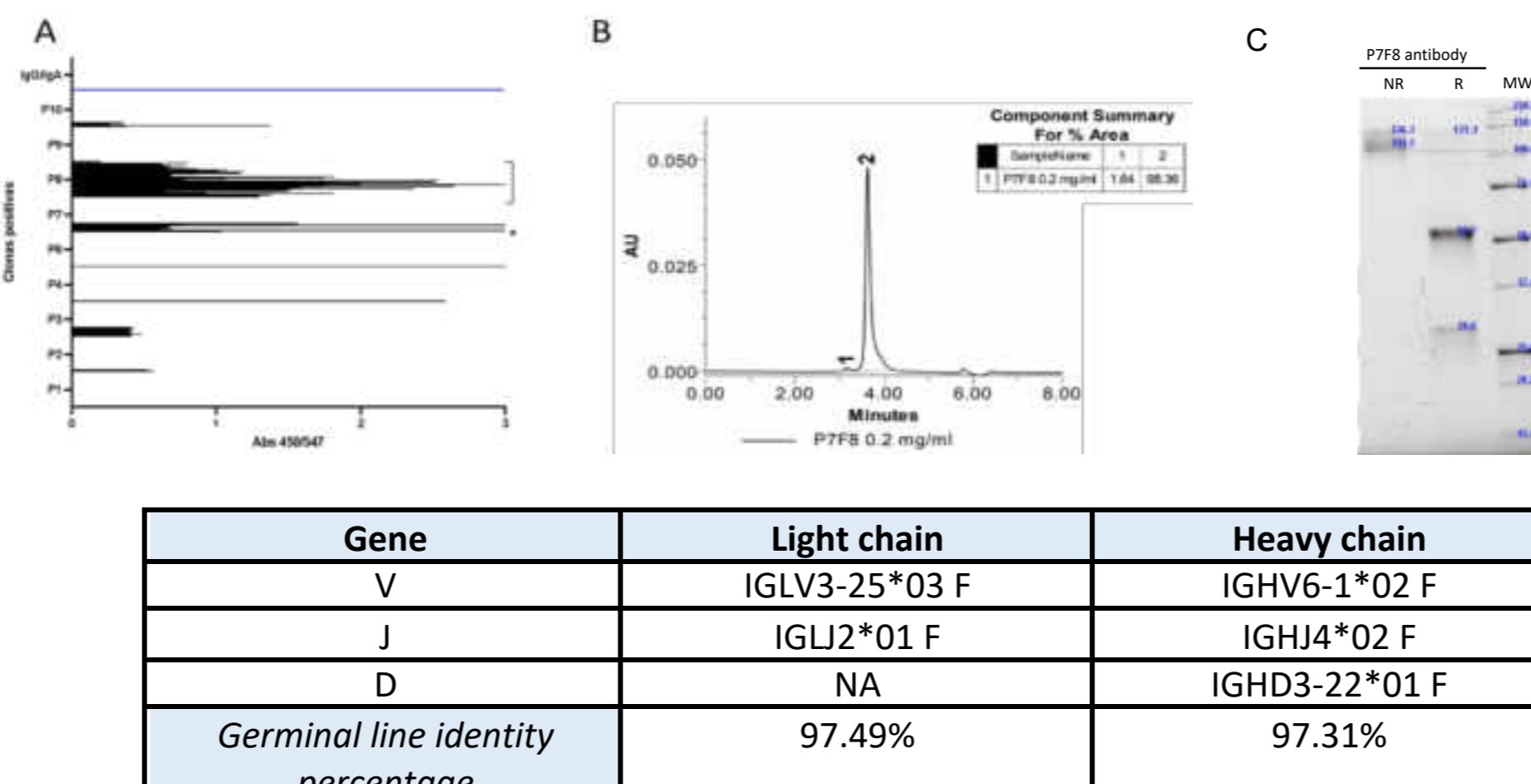


**Figure 3. Sorting of SARS-CoV-2 specific-B cells from two different patients.** The panels show the sequential selection of CD22<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>CD3<sup>+</sup>CD16<sup>+</sup>CD14<sup>+</sup>IgM<sup>+</sup>RBD<sup>+</sup> cells (SARS-CoV-2 specific B-cells sorted) from two different convalescent patients P8 T2 and P9. The PBMCs were isolated and the CD22<sup>+</sup> B cells separated after the MACS microbeads. The cells were sorted with antibodies PE-Cy<sup>5</sup> anti-human CD3 (BD Bioscience Cat. 555334), PerCP-Cy<sup>5</sup>5 anti-human CD14 (BD Bioscience Cat. 550787), PE-Cy<sup>5</sup> anti-human CD16 (BD Bioscience Cat. 555408), PE-Cy<sup>5</sup>7 anti-human CD20 (BD Bioscience Cat. 335793), FITC anti-human CD27 (BD Biosciences Cat 5555440), APC anti-human IgM (BD Biosciences Cat 551062) and PE anti-FC IgG mouse:RBD-Fc mouse.

### Selection and proliferation of the Lymphoblastoid cell lines (LCL) that produce antibodies against SARS-CoV-2 RBD

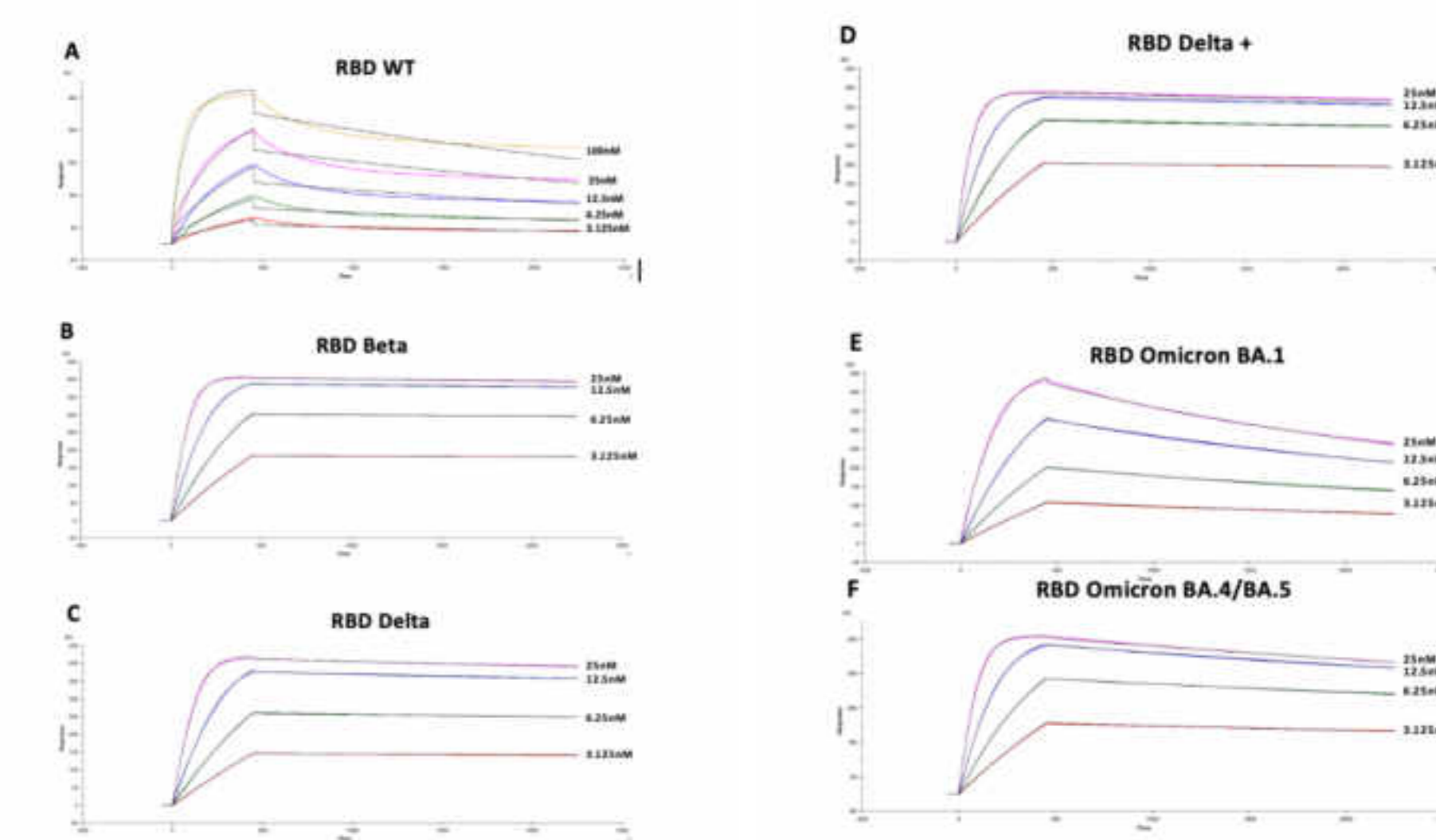


**Figure 4. Proliferation of lymphoblastoid cell lines through the time.** Representative images of the lymphoblastoid cell lines morphology obtained from blood samples of convalescent patients in different times after the immortalization with Epstein Barr virus. The images were obtained with an inverted microscope and the support with AmScope program.



**Figure 4. Evaluation of SARS-CoV-2 RBD antibodies** A) the supernatant of lymphoblastoid cell lines from the convalescent patient was evaluated by indirect ELISA assay. The signal of the positive control IgA (patient serum 1:100) and IgG (patient serum 1:100) is shown in blue. The P7F8 antibody is marked with asterisk. B) The chromatographic profile of the P7F8 antibody is presented after its purification with a Protein A column. C) The molecular weight of the P7F8 antibody in non-reducing versus reducing conditions is shown. The comparison of the DNA sequences of the light and heavy chains of the P7F8 antibody with their closest related germline sequences is also presented (IMGT/V-QUEST).

### P7F8, an IgG antibody that recognizes with high affinity all the SARS-CoV-2 RBD variants of concern



Variant of Concern	SPR			ELISA
	kon (M <sup>-1</sup> s <sup>-1</sup> )	koff (s <sup>-1</sup> )	KD (nM)	EC <sub>50</sub> (nM)
RBD WT	1.173 x 10 <sup>5</sup>	2.411 x 10 <sup>-4</sup>	2.056	1.89
RBD Beta (B.1.351)	7.285 x 10 <sup>5</sup>	1.329 x 10 <sup>-5</sup>	0.018	2.32
RBD Delta (B.1.617.2)	6.383 x 10 <sup>5</sup>	3.277 x 10 <sup>-5</sup>	0.051	3.52
RBD Delta Plus (B.1.617.2 + K417N)	8.090 x 10 <sup>5</sup>	3.193 x 10 <sup>-5</sup>	0.039	3.27
RBD Omicron (BA.1)	3.861 x 10 <sup>5</sup>	3.398 x 10 <sup>-4</sup>	0.880	0.15
RBD Omicron (BA.4/BA.5)	6.888 x 10 <sup>5</sup>	1.026 x 10 <sup>-4</sup>	0.148	1.46

**Figure 6. The P7F8 antibody binding to different SARS-CoV-2 RBD variants of concern determined by Surface Plasmon Resonance and ELISA.** Representative sensorgrams of the interaction of RBD WT (A), RBD Beta (B), RBD Delta (C), RBD Delta plus (D), RBD Omicron BA.1 (E), RBD Omicron BA.4/BA.5 (F) with the P7F8 antibody. Kinetic data were fit to the 1:1 binding model. The association (kon), dissociation (koff), and equilibrium dissociation (KD) constants are presented. RU, resonance units. Comparison of KD obtained in real time by SPR and ELISA (EC<sub>50</sub>) are also shown.

## CONCLUSIONS

- The immortalization of SARS-CoV-2 specific B-cells from convalescent patients was optimized to obtain lymphoblastoid cell lines (LCL) that produce antibodies IgG or IgA.
- We have obtained hundreds of SARS-CoV-2 specific lymphoblastoid cell lines, several of them produce antibodies that recognizes the SARS-CoV-2 RBD and some variants of concern.
- The P7F8 LCL produces in culture cell an IgG monomeric, with a yield of 12.5mg/L.
- The P7F8 antibody recognizes the principal SARS-CoV-2 variants of concern with subnanomolar affinity.
- The P7F8 IgG antibody has the IGHJ3-22 germline, that encoding the YYDRxG motif, which has been involved in the neutralization of SARS-CoV-2 and its variants of concern.

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