SUPPLEMENTARY MATERIALS

Relative Ratios of Human Seasonal Coronavirus Antibodies Predict the Efficiency of Cross-Neutralization of SARS-CoV-2 Spike Binding to ACE2

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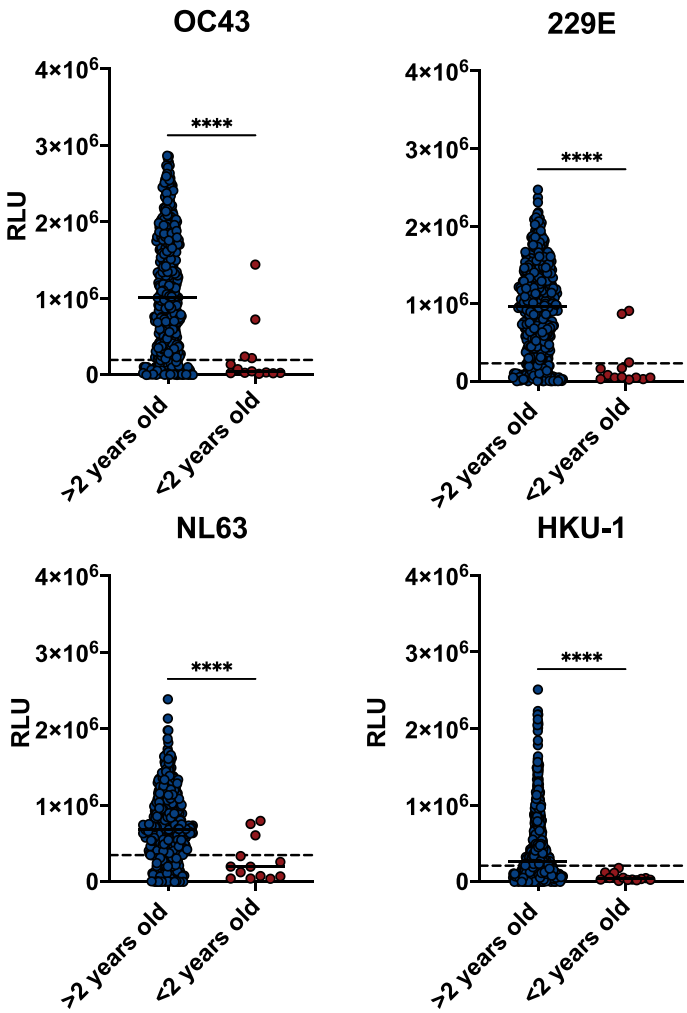
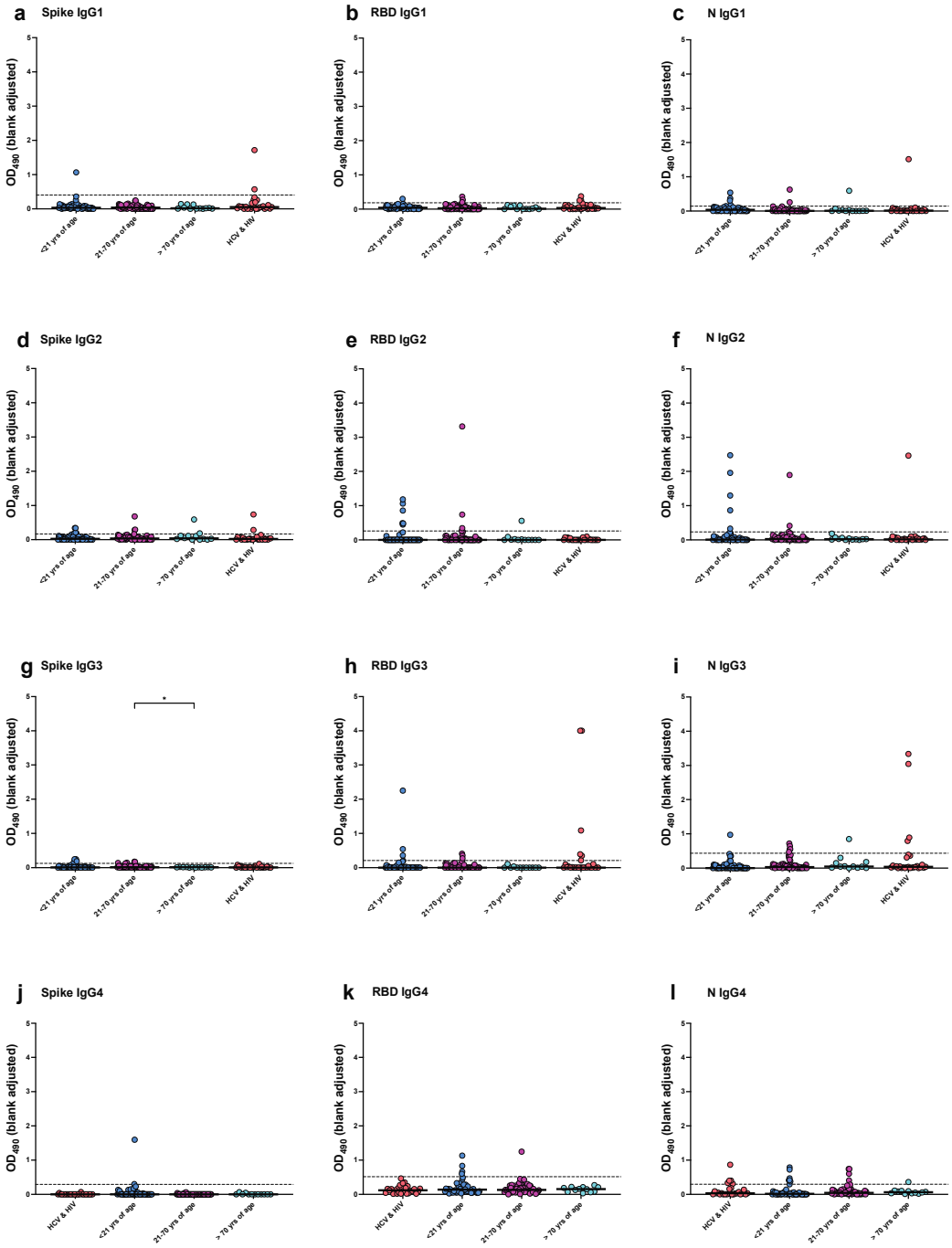
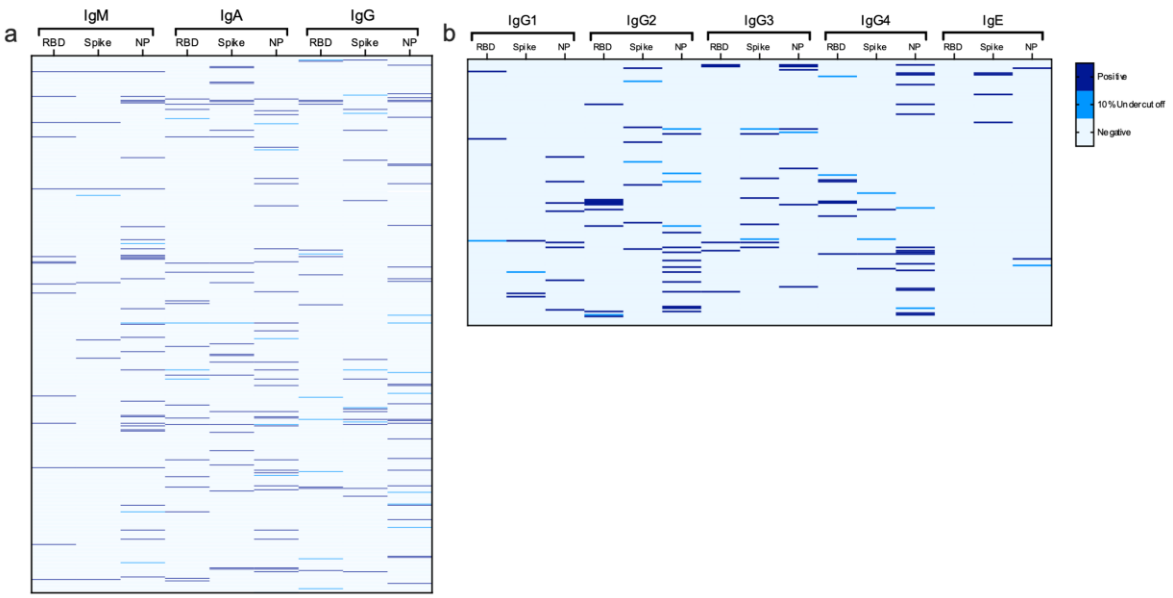


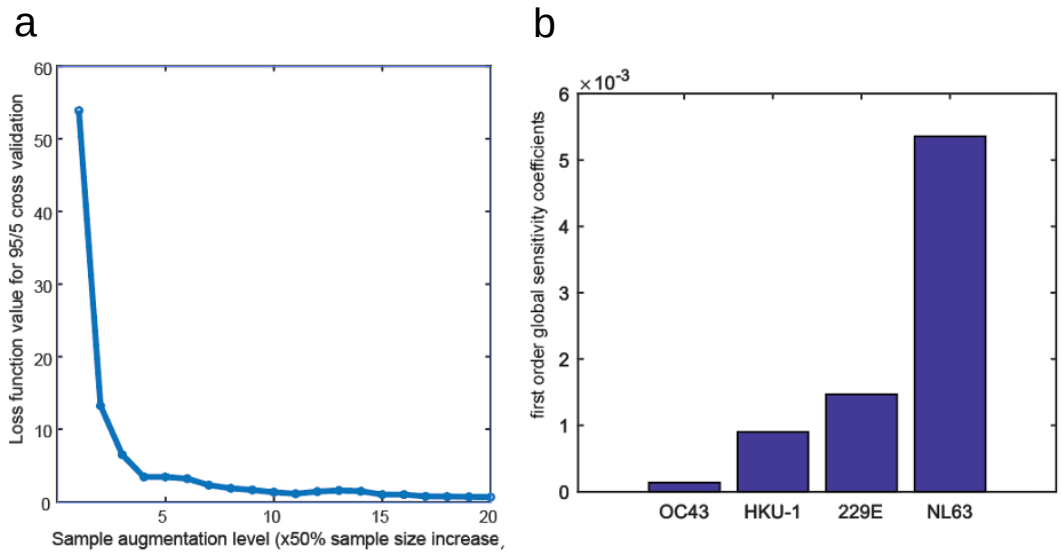
Supplementary Figure 1. Comparison between pediatric samples under 2 years old to the complete cohort for each seasonal spike coronavirus. Pediatric individuals under two years old that were used to establish the cut-offs are indicated (red) in contrast to the seroprevalence of the combination of the four cohorts (Blue). <2 yrs of age [n=13], >2 yrs of age [n=550]. Welch's unpaired T test was performed to establish statistical significance *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplementary Figure 2. IgG-subclass antibody responses to SARS-CoV-2 antigens. The pre-COVID-19 patient cohort samples (x-axis) of HCV & HIV [n=28]; **< 21 yrs,** n=[53]; **21 - 70 yrs** [n=68]; **> 70 yrs** [n=12]; were tested for the reactivity of IgG1 IgG2 IgG3 and IgG4 to the SARS-CoV-2 S-trimer protein **(a, d, g, j)** S-RBD **(b, e, h, k)** and nucleocapsid **(c, f, i ,l)**. The ELISA OD_{490} values indicates the raw OD subtracted from the background. Cut-off values calculated by two rounds of exclusion at 2 Standard deviation from the negative distribution was indicated on each graph. Statistics were one-way ANOVA with Welch correction, *post-hoc* tests were Games-Howell's multiple comparison tests *p<0.05, **p<001, ***p<0.001, ****p<0.0001.



Supplementary Figure 3. Discordance of serological false positive per isotype and antigen. (a) An alignment of the signal to cut-off ratio for each sample was performed by isotype (IgG, IgM, IgA) and antigens (S-RBD, S-Trimer, N) on 589 samples from which 9 were longitudinal. Values over cut-offs (dark blue) and 10% under cut offs (light blue) are indicated. **(b)** An alignment of the signal to cut-off ratio for was performed by subtypes (IgG1, IgG2, IgG3, IgG4) and IgE and antigens (RBD, Spike, N) on 161 randomly selected samples.



Supplementary Figure 4. Global sensitivity analysis (GSA) results for the Gaussian process regression (GPR) model. **(a)** The effect of sample size increase was determined using up-sampling with SMOTE algorithm on the loss function in the cross-validation study of our GPR model showing major increase in cross validation accuracy (reduced Loss function) with sample size increase. Loss function is calculated as: $L = \sum_{j=1}^{n} w_j I\{y_j^{model} \neq y_j\}$ where w_j is the weight for observation j normalized so that they sum to the corresponding prior class probability. **(b)** GSA showing the contribution of the uncertainty of each input factor on the uncertainty of the output. (Calculations were performed using library FLAX running under Matlab (2021). Global Sensitivity Analysis Toolbox ; MATLAB Central File Exchange. Retrieved September 25, 2021). Shown are first order global sensitivity coefficients calculated for 30,000 samples for the quasi-random Monte Carlo sampling calculated using Fourier amplitude sensitivity test (FAST) (Saltelli, A., and Bolado, R. (1998). An alternative way to compute Fourier amplitude sensitivity test (FAST). *Comput. Stat. Data Anal.* 26, 445–460. doi: 10.1016/S0167-9473(97)00043-1).

Table S1. Demographic information of post-pandemic controls. Serum samples collected in various longitudinal studies of SARS-CoV-2 infected and non-infected individuals were used as reference groups. Demographic information was collected from the respective study or patient file and compiled.

Cohorts	SARS-CoV-2 status	# of Subjects	Age range (yrs)	Female % (Mean Age ± SD)	Male % (Mean Age ± SD)
SARS-CoV-2 convalescent	Positive	45	23-72	73 (49 ±13 yrs)	27 (45 ±13 yrs)
SARS-CoV-2 negative	Negative	115	22-73	68 (42 ±12 yrs)	32 (44 ±12yrs)
SARS-CoV-2 11-74 days post PCR	Positive	9	24-90	33 (62 ± 15yrs)	67 (60 ± 25 yrs)
SARS-CoV-2 1-9 days post PCR	Positive	9	46-88	44 (72 ± 18yrs)	56 (68 ± 13yrs)

SD: standard deviation

Table S2. Determination of SARS-CoV-2 assay cut-off values of the four cohorts combined and false discovery rate (FDR) per antigen, isotype & subtypes. Cut-off values were calculated from two exclusion cycles at 2 standard deviations from the negative distributions. Any sample above that designated cut-off was included to determine the FDR

SARS-CoV-2 antigen	Anti-human antibodies	Cut-off	False discovery rate (%)
RBD	IgA	0.428	6.45
	IgM	0.243	5.60
	IgG	0.240	4.58
	IgG1	0.186	4.35
	IgG2	0.262	6.83
	IgG3	0.207	7.45
	IgG4	0.517	3.11
	IgE	0.278	0.59
S	IgA	0.670	4.92
	IgM	0.290	5.60
	IgG	0.357	4.92
	IgG1	0.405	1.86
	IgG2	0.169	8.07
	IgG3	0.125	5.59
	IgG4	0.289	1.86
	IgE	0.230	3.11
N	IgA	0.857	13.24
	IgM	0.617	7.98
	IgG	0.551	11.0
	IgG1	0.143	5.59
	IgG2	0.232	5.59
	IgG3	0.432	6.83
	IgG4	0.294	9.94
	IgE	0.260	1.24

Table S3. Determination of SARS-CoV-2 assay cut-off values of pre-COVID-19 HCV and HIV patients and corresponding false discovery rate per antigen, isotype & subtypes. Cut-off values were calculated from two exclusion cycles at 2 standard deviations from the negative distributions. Any sample above that designated cut-off was included to determine the FDR.

SARS-CoV-2 antigen	Anti-human antibodies	Cut-off	False discovery rate (%)
RBD	IgA	0.428	6.78
	IgM	0.243	5.08
	IgG	0.240	8.47
	IgG1	0.186	10.7
	IgG2	0.262	0.00
	IgG3	0.207	21.4
	IgG4	0.517	0.00
	IgE	0.278	0.00
S	IgA	0.670	13.6
	IgM	0.290	6.78
	IgG	0.357	6.78
	IgG1	0.405	7.14
	IgG2	0.169	7.14
	IgG3	0.125	0.00
	IgG4	0.289	0.00
	IgE	0.230	7.14
N	IgA	0.857	15.3
	IgM	0.617	6.78
	IgG	0.551	15.3
	IgG1	0.143	3.57
	IgG2	0.232	3.57
	IgG3	0.432	14.3
	IgG4	0.294	17.9
	IgE	0.260	3.57

Table S4. Determination of SARS-CoV-2 assay cut-off values of pre-COVID-19 > 70 years of age and corresponding false discovery rate per antigen, isotype & subtypes. Cut-off values were calculated from two exclusion cycles at 2 standard deviations from the negative distributions. Any sample above that designated cut-off was included to determine the FDR.

SARS-CoV-2 antigen	Anti-human antibodies	Cut-off	False discovery rate (%)
RBD	IgA	0.428	6.25
	IgM	0.243	3.13
	IgG	0.240	1.56
	IgG1	0.186	0.00
	IgG2	0.262	8.33
	IgG3	0.207	0.00
	IgG4	0.517	0.00
	IgE	0.278	8.33
S	IgA	0.670	1.56
	IgM	0.290	0.00
	IgG	0.357	1.56
	IgG1	0.405	0.00
	IgG2	0.169	16.7
	IgG3	0.125	0.00
	IgG4	0.289	0.00
	IgE	0.230	0.00
N	IgA	0.857	21.9
	IgM	0.617	6.25
	IgG	0.551	14.1
	IgG1	0.143	8.33
	IgG2	0.232	0.00
	IgG3	0.432	8.33
	IgG4	0.294	8.33
	IgE	0.260	0.00

Table S5. Determination of SARS-CoV-2 assay cut-off values of pre-COVID-19 21 - 70 years of age and corresponding false discovery rate per antigen, isotype & subtypes. Cut-off values were calculated from two exclusion cycles at 2 standard deviations from the negative distributions. Any sample above that designated cut-off was included to determine the FDR.

SARS-CoV-2 antigen	Anti-human antibodies	Cut-off	False discovery rate (%)
RBD	IgA	0.428	5.86
	IgM	0.243	4.40
	IgG	0.240	3.66
	IgG1	0.186	4.41
	IgG2	0.262	5.88
	IgG3	0.207	4.41
	IgG4	0.517	1.47
	IgE	0.278	0.00
S	IgA	0.670	5.86
	IgM	0.290	3.30
	IgG	0.357	4.40
	IgG1	0.405	0.00
	IgG2	0.169	4.41
	IgG3	0.125	5.88
	IgG4	0.289	0.00
	IgE	0.230	4.41
N	IgA	0.857	15.8
	IgM	0.617	12.8
	IgG	0.551	12.5
	IgG1	0.143	2.94
	IgG2	0.232	4.41
	IgG3	0.432	7.35
	IgG4	0.294	5.88
	IgE	0.260	0.00

Table S6. Determination of SARS-CoV-2 assay cut-off values of pre-COVID 19 < 21 years of age and corresponding false discovery rate per antigen, isotype & subtypes. Cut-off values were calculated from two exclusion cycles at 2 standard deviations from the negative distributions. Any sample above that designated cut-off was included to determine the FDR.

SARS-CoV-2 antigen	Anti-human antibodies	Cut-off	False discovery rate (%)
RBD	IgA	0.428	7.25
	IgM	0.243	8.29
	IgG	0.240	5.70
	IgG1	0.186	1.89
	IgG2	0.262	11.3
	IgG3	0.207	5.66
	IgG4	0.517	7.55
	IgE	0.278	0.00
S	IgA	0.670	2.07
	IgM	0.290	10.4
	IgG	0.357	6.22
	IgG1	0.405	1.89
	IgG2	0.169	11.3
	IgG3	0.125	9.43
	IgG4	0.289	3.77
	IgE	0.230	0.00
N	IgA	0.857	6.22
	IgM	0.617	2.07
	IgG	0.551	6.74
	IgG1	0.143	9.43
	IgG2	0.232	9.43
	IgG3	0.432	1.89
	IgG4	0.294	11.3
	IgE	0.260	1.89

Table S7 Multi-antigen FDR calculation. False discovery rate of all four cohorts combined using a single antigen, combinations of two antigens and three antigens were compared by each isotype and subtype.

Antibody Isotype	Single antigen FDR (%)			Two antigen FDR (%)			Three antigen FDR (%)
	RBD	Spike	N	RBD + S	RBD + N	S + N	RBD + S+N
IgA	6.45	4.92	13.2	1.19	1.87	1.70	0.68
IgM	5.60	5.60	7.98	2.21	1.19	0.68	0.51
IgG	4.58	4.92	11.0	1.53	1.53	0.85	0.51
IgG1	4.35	1.86	5.59	0.62	0.62	0.00	0.00
IgG2	6.83	8.07	5.59	0.62	0.62	0.00	0.00
IgG3	7.45	5.59	6.83	0.62	1.86	0.62	0.00
IgG4	3.11	1.86	9.94	0.62	0.62	0.62	0.62
IgE	0.62	3.11	1.24	0.00	0.00	0.00	0.00

Table S8. Demographics of fuzzy mean clusters of patients segregated by serum antibody cross-reactivity. Cross-reactivity against 3 different SARS-CoV-2 antigens (N, RBD, Spike) for IgA, IgM and IgG were included with capacity of these antibodies to inhibit Spike-ACE2 binding.

Cluster # (n)	Age Mean ± SD (Range)	Sex (% Female)
1 (79)	41±26 (1-90 yrs)	58
2 (21)	32±23 (3-69 yrs)	42
3 (16)	45±24 (4-68 yrs)	38
4 (36)	45±26 (1-79 yrs)	44
5 (86)	45±26 (1-90 yrs)	47
6 (1)	21 yrs	0
7 (53)	41±28 (2-80 yrs)	42
8 (89)	35±28 (1-90 yrs)	47
9 (65)	35±28 (2-96 yrs)	57
10 (60)	36±28 (1-97 yrs)	50

Total n = 507*; *Subject 137-48 (56 yrs old, Female from HCV&HIV cohort) was weakly clustered in multiple clusters and was not associated with a single group. This sample was excluded from the analysis.