

Supplement to “Severe Acute Respiratory Syndrome Coronavirus 2 Receptor (Human Angiotensin-Converting Enzyme 2) Binding Inhibition Assay: A Rapid, High-Throughput Assay Useful for Vaccine Immunogenicity Evaluation”

Table S1. Assay robustness - lower incubation time limit results

Sample #	Sample ID	Reference Condition Titer	Lower Incubation Time Limit		% Recovery	% Difference
			Titer ^a	GMT		
1	HQC	158.5	148 135.4	141.6	89.3	-10.7
2	MQC	66.6	61.4 60.5	60.9	60.9	91.5
3	LQC	37.6	35.8 35.4	35.6	35.6	94.7
4	NC	5	5 5	5	5	100
5	Serum #12	11	8.7 8.4	8.5	8.5	77.7
6	Serum #13	115.5	101.5 103.9	102.7	102.7	88.9
7	Serum #14	22.3	19.5 18.6	19	19	85.4
8	Serum #15	170.3	149.3 166.2	157.5	157.5	92.5
9	Serum #16	54.7	48.6 46.2	47.4	47.4	86.6
10	Serum #17	93.7	76	76.7	76.7	81.9

			77.4			
11	Serum #18	22.4	21.8	21	21	93.9
			20.3			
12	Serum #19	61.6	50.3	51.3	51.3	83.3
			52.4			
13	Serum #20	18	19.1	17.7	17.7	98.3
			16.4			
14	Serum #21	26.9	26.2	26.3	26.3	98
			26.5			
15	Serum #22	27.9	26.1	26.3	26.3	94.4
			26.6			
16	Serum #23	62.3	52.3	55.1	55.1	88.4
			58			
17	Serum #24	13.6	14.9	14	14	103.1
			13.2			
18	Serum #1	91.4	91.5	88.4	88.4	96.8
			85.5			
19	Serum #2	62.4	54.9	58.5	58.5	93.7
			62.3			
20	Serum #3	81.1	88.3	90.2	90.2	111.3
			92.2			

*Samples were tested in duplicate in each assay, so 2 titer values are shown for each sample.

Numbers in italics indicate values outside of acceptable range.

GMT—geometric mean titer; HQC—high quality control; ID—identification; LQC—low quality control; MQC—mid quality control; NC—negative control.

Table S2. Assay robustness - upper incubation time limit results

Sample #	Sample ID	Reference Condition Titer	Upper Incubation Time Limit		% Recovery	% Difference
			Titer ^a	GMT		
1	HQC	158.5	149.4 158.7	154	97.1	-2.9
2	MQC	66.6	64.4 61.5	62.9	94.5	-5.5
3	LQC	37.6	39 39.6	39.3	104.5	4.5
4	NC	5	5 5	5	100	0
5	Serum #12	11	10.3 11.4	10.8	98.5	-1.5
6	Serum #13	115.5	114.4 112.6	113.5	98.3	-1.7
7	Serum #14	22.3	23.8 25.1	24.4	109.6	9.6
8	Serum #15	170.3	161.7 190.8	175.6	103.1	3.1
9	Serum #16	54.7	59 61.5	60.2	110.1	10.1
10	Serum #17	93.7	95.5 93.7	94.6	101	1
11	Serum #18	22.4	27 27.9	27.4	122.5	22.5

12	Serum #19	61.6	63.6	74	120.2	20.2
			86.2			
13	Serum #20	18	19.1	20.1	111.8	11.8
			21.2			
14	Serum #21	26.9	28.7	30.1	111.8	11.8
			31.5			
15	Serum #22	27.9	31	30.5	109.3	9.3
			30			
16	Serum #23	62.3	61	65.5	105.2	5.2
			70.4			
17	Serum #24	13.6	16.3	16.1	118.4	18.4
			15.9			
18	Serum #1	91.4	97.6	93.6	102.4	2.4
			89.7			
19	Serum #2	62.4	61	61.2	98.2	-1.8
			61.5			
20	Serum #3	81.1	117.9	133.8	165	65
			151.8			

^aSamples were tested in duplicate in each assay, so 2 titer values are shown for each sample.

Numbers in italics indicate values outside of acceptable range.

GMT—geometric mean titer; HQC—high quality control; ID—identification; LQC—low quality control; MQC—mid quality control; NC—negative control.

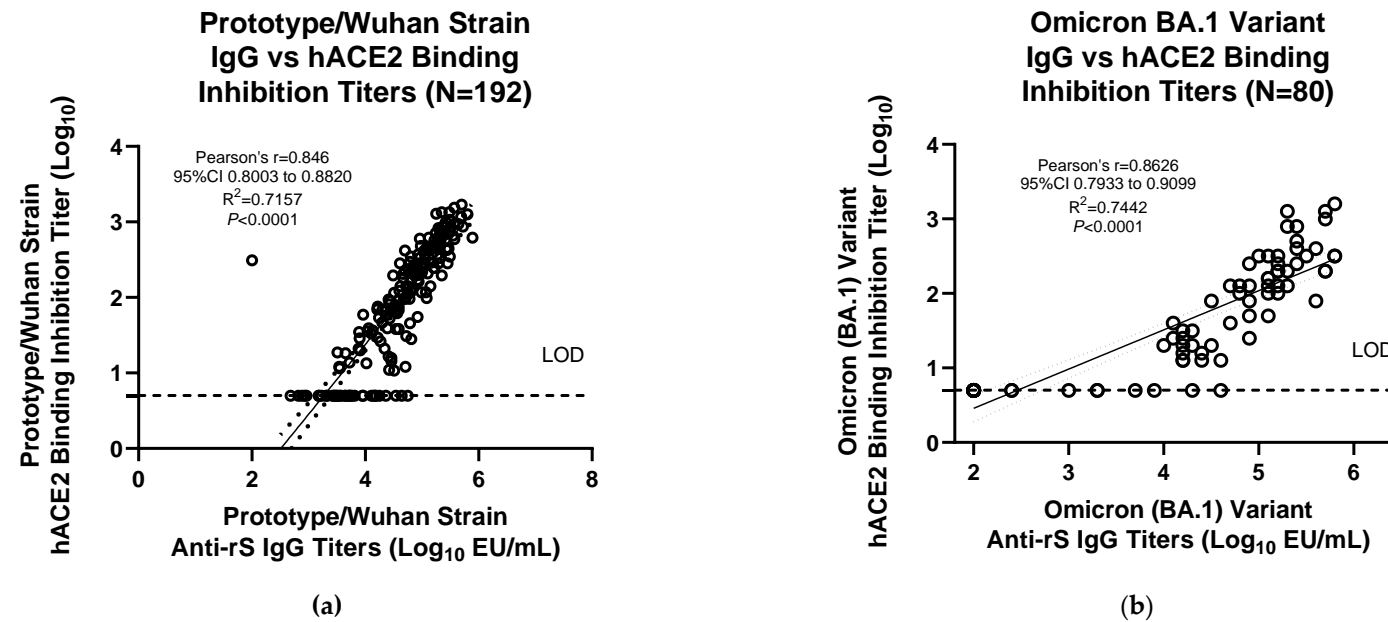


Figure S1. Correlation of prototype/Wuhan strain and Omicron variant (BA.1) hACE2 binding inhibition with anti-rS IgG. **(a-b)** Detection of anti-rS IgG antibodies was performed as previously described in Formica et al. 2021 [18]. Linear regression analysis was performed with all the hACE2 binding inhibition results, including values at LOD, to compare results from the IgG assay and the hACE2 binding inhibition assay for **(a)** prototype/Wuhan strain or **(b)** Omicron BA.1 variant. hACE2—human angiotensin-converting enzyme 2; IgG—immunoglobulin G; LOD— limit of detection; Log_{10} —logarithm with base 10; rS—recombinant spike.