Parameter Comparison For Three Assays To Perform Antigen-Specific Antibody Biomarker Discovery Against 8 Antigens, 4 probes For 14 Samples With 3 Repeats			
	LC-MS	Traditional ELISA	µMAP
Multiplexed (target antigen & probe)	No	No	Yes
Glycosylation Measurement	Yes	No	Yes
How Is Glycosylation Quantified?	Direct detection by Mass Spectrum	N/A	Indirectly by lectin probe binding
Samples per assay run	1	1/plate	14/slide
Total Serum volume before concentration/dilution (µl)	4800-12000	48 (diluted to 4800)	0.15 (diluted to 15)
Runtime since sample addition for one assay (hr)	6	2.5	2
Total Assay Runtime to Test All Samples (hr)	336	35	<3
Equipment Cost (k$)	200-889	25	253
Assay Unit Cost ($)	250	3	10
Dynamic Range (log)	4-6[1]	2-3[2]	>3

Table 1: Comparison between existing methods and current work.

% S Label of Sialic Acid-Modified Glycoengineered Serum Sample	Actual Sialic Acid % Among Glycans In The Serum Sample
100	9.3258315
75	7.8632774
50	6.0212389
25	3.5244399
0	2.6262206

Table S2: Comparison between numerical value of % S label of sialic acid-modified glycoengineered serum sample and actual sialic acid % among glycans in the serum sample measured by LC/MS. Pearson correlation between the numeric values equals 0.9922, suggesting the %S label we assign is linearly correlated with actual percentage of sialic acid.

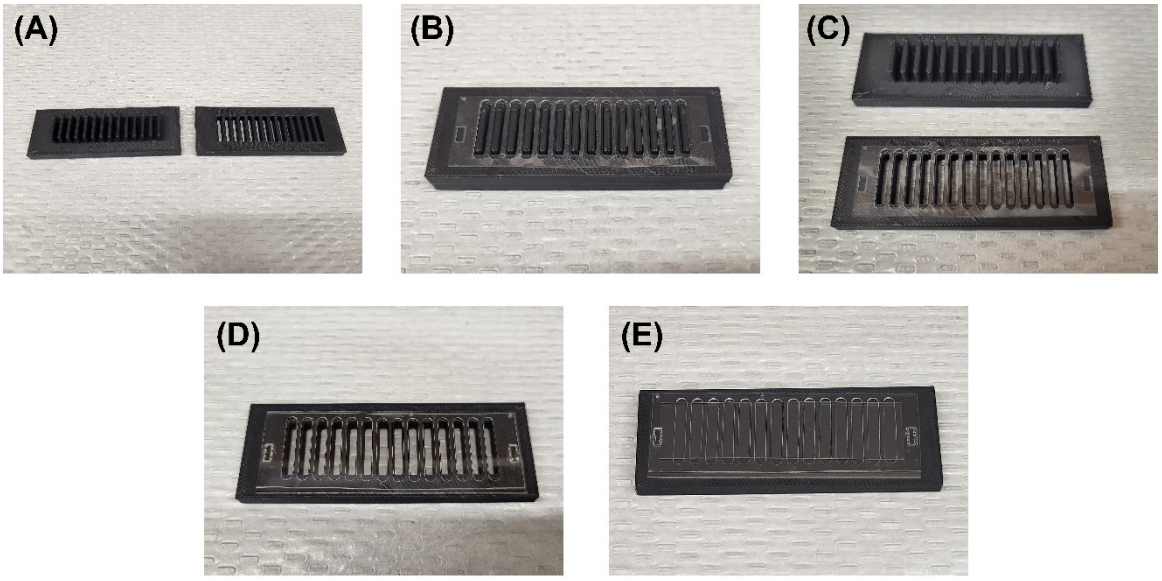

Figure S1: Assembly process of the μMAP when used for sample incubation: A. Two pieces of sample alignment block. B. Stack alignment blocks and PDMS for sample channel together. C. Remove bottom block while keeping the PDMS on top block. D. Press the glass slide on the top of the block, with rectangular marks aligned. E. Put the PDMS for top cover layer on the top of the sample channel layer.

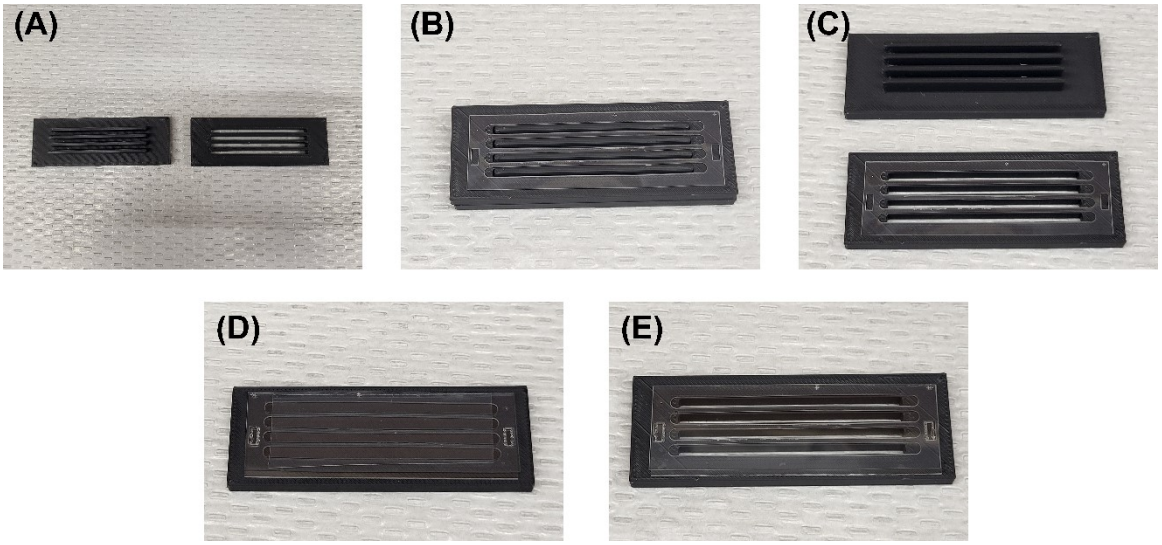

Figure S2: Assembly process of the μMAP when used for probe incubation: A. Two pieces of probe alignment block. B. Stack alignment blocks and PDMS for probe channel together. C. Remove bottom block while keeping the PDMS on top block. D. Press the glass slide on the top of the block, with rectangular marks aligned. E. Put the PDMS for top cover layer on the top of the probe channel layer.

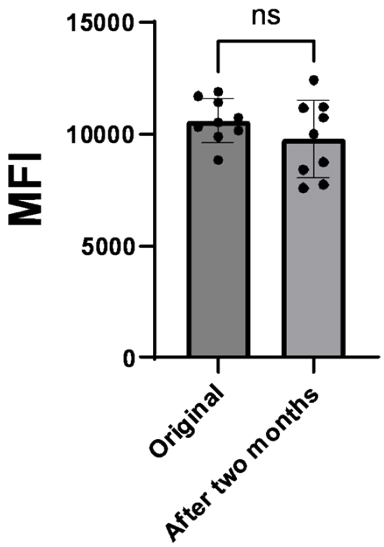

Figure S3: Stability of the microarray slide using the Protein A/G assay shown in Figure 4.A. A slide with protein A/G was stored for two months before running the same A/G assay (N = 9). MFI was compared using an unpaired T test with P = 0.22.

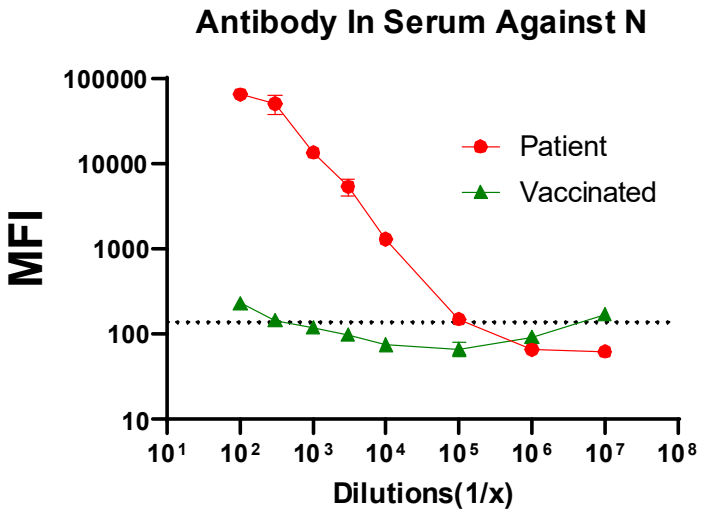

Figure S4: MFI of Sars-CoV-2 nucleocapsid protein specific IgG in individual samples of Sars-CoV-2 patient, and vaccinated individuals with a repeat of N = 2. The dynamic range of 1:300 to 1:10^5 dilution is considered reliable.

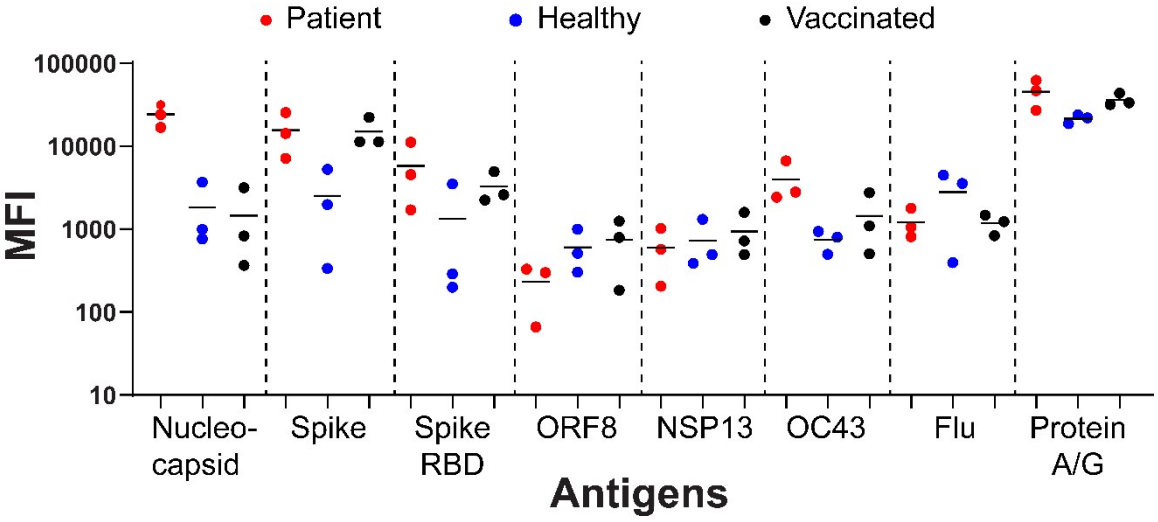

Figure S5: MFI of antigen specific IgG in individual samples of Sars-Cov-2 patient, pre pandemic healthy individuals and vaccinated individuals. 6 Sars-Cov-2 antigens and 2 controls were selected. Each dot represents one individual with a repeat of N = 2.

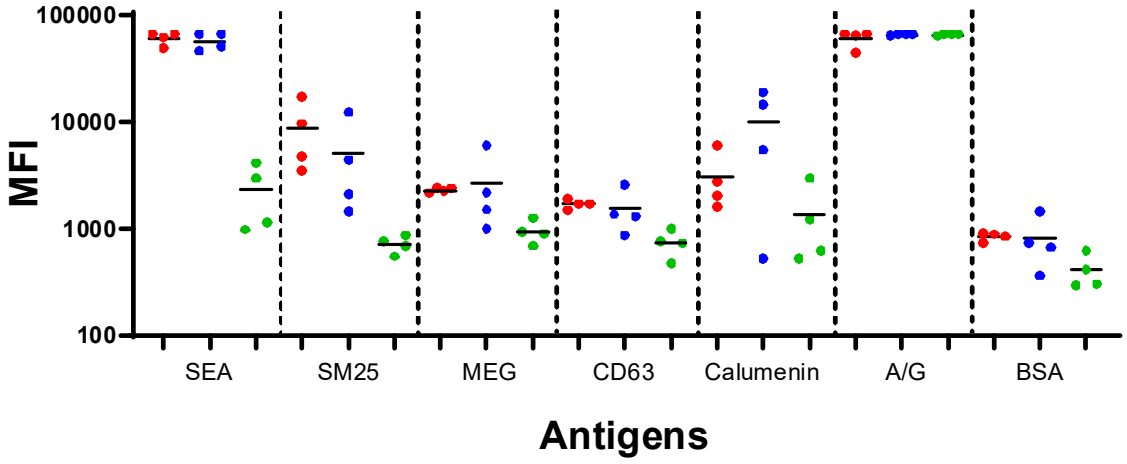

Figure S6: MFI of 5 Schistosomiasis antigen specific IgG in individual samples of SEA+Egg+ (current infection, red), SEA+Egg- (past infection, blue) and SEA-Egg- (healthy, green) from Schistosomiasis-active regions in Brazil. Each dot represents one individual with a repeat of N = 3, except for BSA, whose N = 2.

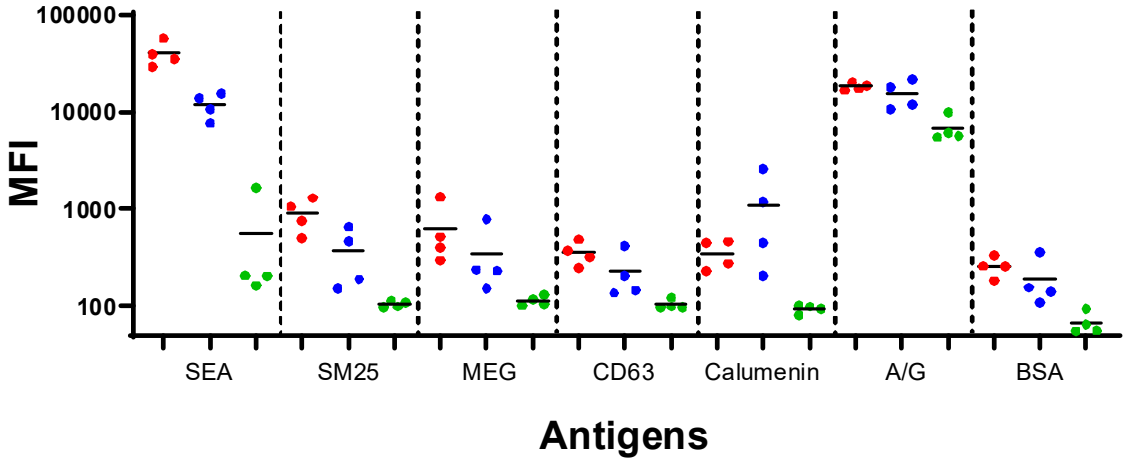

Figure S7: MFI of 5 Schistosomiasis antigen specific IgG4 in individual samples of SEA+Egg+ (current infection, red), SEA+Egg- (past infection, blue) and SEA-Egg- (healthy, green) from Schistosomiasis-active regions in Brazil. Each dot represents one individual with a repeat of N = 3, except for BSA, whose N = 2.

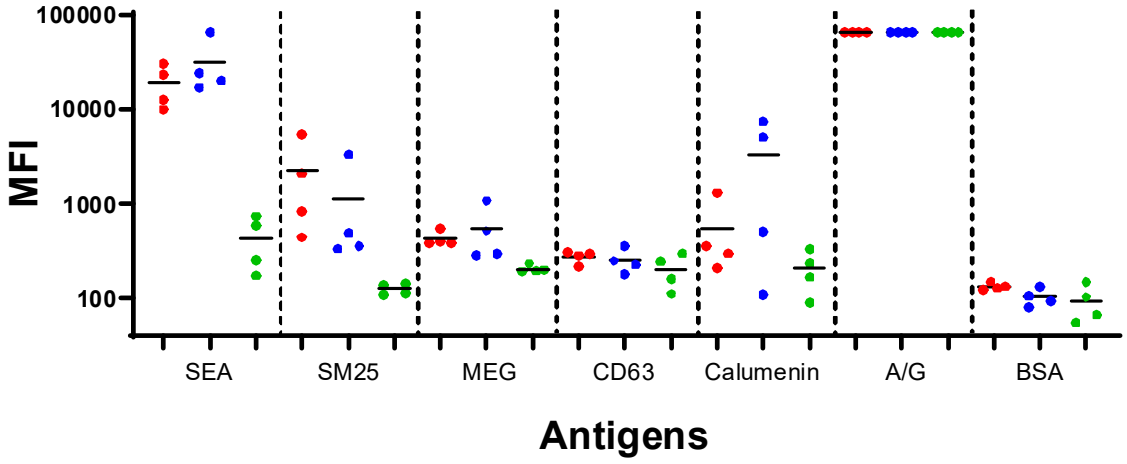

Figure S8: MFI of 5 Schistosomiasis antigen specific FcR2A binding in individual samples of SEA+Egg+ (current infection, red), SEA+Egg- (past infection, blue) and SEA-Egg- (healthy, green) from Schistosomiasis-active regions in Brazil. Each dot represents one individual with a repeat of N = 3, except for BSA, whose N = 2.

References

1. T. E. Angel, U. K. Aryal, S. M. Hengel, E. S. Baker, R. T. Kelly, E. W. Robinson and R. D. Smith, *Chem Soc Rev*, 2012, **41**, 3912-3928.
2. A. Online, SARS-CoV-2 / COVID-19 ELISA Kits, https://www.antibodies-online.com/areas/infectious-disease/covid-19/sars-cov-2-elisa-kits/?srsltid=AfmBOorVASvAKd3vtdgTOXQmxkoWa-b4gsaqUlCswmfnBTrMBsZI2WUk, (accessed 02/18, 2025).