

TOP-Plus is a Versatile Biosensor Platform for Monitoring SARS-CoV-2 Antibody Durability

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Abbreviation list

BLI, Bio-Layer Interferometry; COVID-19, Corona Virus Disease-2019; dR, relative dissociation rate; ELISA, Enzyme-linked immunosorbent assays; HAMA, human anti-mouse antibodies; HPLC, high performance liquid chromatography; PRNT, plaque reduction neutralization test; PsV, pseudo virus neutralization test; RBD, receptor-binding domain; RFU, relative fluorescence unit; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SNAbs, surrogate neutralizing antibody; SPR, Surface Plasmon Resonance; SRID, single radial immunodiffusion; TAb, total antibody; TOP, testing-on-a-probe; WHO, World Health Organization

Abstract

Background: Low initial SARS-CoV-2 antibody titers dropping to undetectable levels within months after infection have raised concerns over long term immunity. Both the antibody levels and avidity of the antibody-antigen interaction should be examined to understand the quality of the antibody response.

Methods: A testing-on-a-probe “plus” panel (TOP-Plus) was developed, which included a newly developed avidity assay built into the previously described SARS-CoV-2 TOP assays that measured total antibody (TAb), surrogate neutralizing antibody (SNAb), IgM and IgG on a versatile biosensor platform. TAb and SNAb levels were compared with avidity in previously infected individuals at 1.3 and 6.2 months post-infection in paired samples from 80 COVID-19 patients. Sera from SARS-CoV-2 vaccinated individuals were also evaluated for antibody avidity.

Results: The newly designed avidity assay in this TOP panel correlated well with a reference Bio-Layer Interferometry avidity assay ($r=0.88$). The imprecision of the TOP avidity assay was less than 10%. Although TAb and neutralization activity (by SNAb) decreased between 1.3 and 6.2 months post-infection, the antibody avidity increased significantly ($P < 0.0001$). Antibody avidity in 10 SARS-CoV-2 vaccinated individuals (median 28 days post-vaccination) was comparable to the measured antibody avidity in infected individuals (median 26 days post-infection).

Conclusion: This highly precise and versatile TOP-Plus panel with the ability to measure SARS-CoV-2 TAb, SNAb, IgG and IgM antibody levels and avidity of individual sera on one sensor can become a valuable asset in monitoring not only SARS-CoV-2-infected patients, but also the status of individuals' COVID-19 vaccination response.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to crippling levels of morbidity and mortality around the world. (1) Seroprevalence studies have begun to show a larger extent of SARS-CoV-2 infections than initially reported because of the high prevalence of infected individuals with mild or no symptoms.(2, 3) However, lower SARS-CoV-2 IgG antibody levels have been reported in those with mild or no symptoms when compared to those with severe COVID-19.(4-7) Furthermore, emerging evidence suggests that SARS-CoV-2 antibodies in some asymptomatic carriers may diminish over time to levels below detection.(8-10) This decrease in antibody levels over time may include neutralizing SARS-CoV-2 antibodies, which play a vital role in viral clearance.(11) These observations raise the question of whether acquired immunity may be short lived and *herd immunity* protection may be less durable than anticipated.(12)

While many studies focus on overall antibody titers, other factors are likely equally important in evaluating the humoral antibody response. Binding titers are determined by the antibody concentration and average affinity. Avidity can be defined as the strengthening of antibody binding through bi- or multivalency or as the functional affinity of the entire IgG, IgA, or IgM molecule, a net product of the intrinsic paratope-epitope affinity and the valency.(13) In the following we use the term *avidity* in the latter sense. Low avidity antibodies are typically produced early in the humoral immune response. (14, 15) Over time, with affinity maturation, the intrinsic affinity of the antibody-antigen interaction strengthens, and so does the functional affinity or avidity of bivalent IgG or classes of higher valency.

To evaluate whether these reported weak early antibody responses should be of clinical concern, various assays have emerged to help assess antibody avidity in the evaluation of the SARS-CoV-2 immune response. (16-19) Antibody avidity may be measured in a variety of ways, including enzyme-linked immunosorbent assays, high performance liquid chromatography, capillary electrophoresis or single radial immunodiffusion. Although providing some insight into the functional affinity, these assays are often qualitative, labor intensive, low-throughput and display low accuracy and precision. Therefore, biosensor technologies such as Surface Plasmon Resonance and Bio-Layer Interferometry (BLI) have become popular in monitoring the molecular binding between antigen and antibody in a real-time and cost effective manner.(20)

This study describes a similar, yet novel, approach to evaluating the level and avidity of SARS-CoV-2 receptor-binding domain (RBD) antibodies using a testing-on-a-probe (TOP)-Plus panel (TOP-Plus) which includes a newly developed avidity assay and the previously described SARS-CoV-2 TOP assays (total antibody [TAb], surrogated neutralizing antibody [SNAbs]) on a single versatile biosensor platform. This fully automated assay panel was used in the current study to evaluate and describe the antibody response and antibody avidity approximately 1 month and 6 months post symptom onset in 80 individuals previously diagnosed with COVID-19. (21) The antibody avidity in 10 vaccinated individuals approximately 1 month post SARS-CoV-2 vaccination (1st dose) was also evaluated as an early demonstration of its use for monitoring the response to vaccination.

Materials and methods

Study participants and source of specimen

The details of participant characteristics and associated COVID-19 symptoms have been described previously. (21, 22) In summary, 80 adults aged 18-76 years who had been diagnosed with SARS-CoV-2 infection or had a confirmed SARS-CoV-2 exposure had blood specimen collected circa 1.3 and circa 6.2 months post infection at the Rockefeller University Hospital. Weill Cornell Medicine performed the antibody analyses as described below (Also see the online Supplemental Material).

Additional blood specimens were collected 1/2/2021-1/28/2021 from a separate cohort of 10 SARS-CoV-2 vaccinated (Moderna, mRNA-1273 vaccine) individuals. Specimens were collected 25-28 days after administration of the 1st vaccine dose but before the 2nd dose (median 28 days post-vaccination). Samples were analyzed on the TOP-Plus biosensor, as described below.

SARS-CoV-2 antibody avidity assay description

The principle of the SARS-CoV-2 antibody avidity assay is similar to a previously described technology (23) that measured SARS-COV-2 antibodies at the tip of an RBD-coated quartz probe and used a biotinylated RBD and a Cy5-Streptavidin conjugate as the signaling elements. However, the calculated relative dissociation rate (dR) allows for avidity testing in this new assay. (**Figure 1**) In short, a RBD precoated probe is sequentially incubated in microwells containing the sample (to capture SARS-CoV-2 specific antibodies), biotinylated RBD and Streptavidin-Cy5 conjugate along with washes between the incubation steps. After the initial fluorescent signal is measured (Signal₀), the probe with the immobilized immunocomplex enters into repetitive dissociation cycles with multiple incubations in phosphate-buffered saline

with Tween[®]-20 detergent (PBST, pH 7.4) as a dissociation buffer. After each incubation, the fluorescent signal is measured (Signal_t). Ultimately, a dissociation curve is constructed by plotting the normalized fluorescent signal (Signal₀/Signal_t) over time. The dR (1/s) is calculated from a function derived by fitting the dissociation curve assuming first order reaction kinetics.

The dissociation profile represents the rate of antibody dissociation from the RBD-coated probe. For an accurate measurement of relative antibody dissociation rate, a limited but adequate amount of antibody is loaded on the probe surface. Loading higher amounts of antibody, determined by getting a high initial fluorescent signal (Signal₀) over a certain threshold, causes the formation of a packed multi-layer antibody construct on the probe surface. This leads to an inaccurate measurement as antibodies in a packed adsorbed layer cannot freely dissociate. Therefore, antibody packing density affects the dissociation measurement. On the other hand, sensitive measurements require adequate antibody loading as determined by the initial fluorescent signal above a certain level. Therefore, proper antibody loading must be within the proper range for accurate measurement. Samples with high antibody concentrations must be diluted for measurement. The appropriate initial fluorescent signal (which verifies an optimal antibody loading) was practically determined via a titration study to be in the range of 20-615RFU, as discussed below under analytical validation. The dilution factor was determined by measuring the initial fluorescent signal to fall within this proper signal range.

Of note, a lower dR reflects both affinity maturation and multivalent binding development. Either a higher intrinsic binding strength of a paratope to RBD or addition of paratopes to the

antibody structure results in a higher binding strength and a lower dR of a COVID-19 antibody-RBD pair.

SARS-CoV-2 antibody avidity analytical validation

Titration studies were performed to determine the proper range of antibody loading. Serum samples with different TAb levels (1171-10872 undiluted relative fluorescence units [RFU]) were randomly selected from four COVID-19 patients to perform titration studies. The pooled SARS-CoV-2 TAb negative serum was used as diluent. Samples with initial fluorescent signal (Signal_0) in the range of 20-615 RFU showed consistent dR values, independent of the signal or concentration level (**Figure 2**). We considered this fluorescent signal range as an indication for optimal antibody loading. Samples with a high fluorescent signal (Signal_0 > 615RFU) were diluted accordingly for measurement. Samples with a low fluorescent signal (Signal_0 <20 RFU) were identified as unmeasurable.

The SARS-CoV-2 antibody avidity assay was evaluated with 12 different purified antibodies against SARS-CoV-2 that were purchased from various vendors. (online **Supplemental Table 1**) These are recombinant human, rabbit or chimeric monoclonal and polyclonal antibodies of varying avidity levels to the RBD. A dissociation curve was generated utilizing pooled human sera from SARS-CoV-2 negative patients that were spiked with one of five antibodies and measured for avidity. The range of antibody concentrations used in the spike-in experiments are listed in online **Supplemental Table 2**. dRs were determined at varying levels of Signal_0 by spiking the negative pooled sera with one of seven antibodies and measured for avidity.

Avidity Assay Precision and Interference

The imprecision of the avidity assay and interference studies, including cross-reactivity studies, are described in the online **Supplemental Material**.

BLI comparison study

BLI measurements by the Gator (Gator Bio) was used to compare the avidity of 12 different purified COVID-19 antibodies (online **Supplemental Table 1**) to the TOP-Plus avidity assay. Gator koff (dissociation-rate constant) measurement was performed at a fixed 10 ug/ml concentration level in a buffer containing 0.2% BSA and 0.02% Tween®-20. The TOP-Plus avidity assay measurements were performed using COVID-19 negative pooled serum spiked with one of these antibodies at a concentration level between 1-30 ug/ml (dependent on the appropriate fluorescence signal, range of 20-615 RFU, as described previously).

SARS-CoV-2 total antibody and surrogate neutralizing antibody assays

The SARS-CoV-2 total antibody TAb and SNAbs assays were used to measure plasma TAb and SNAbs antibodies against SARS-CoV-2. Plasma samples were assayed on the fully automated Pylon 3D analyzer (ET HealthCare), as previously described.(23, 24) Additional information may be found in the online **Supplemental Material**.

Statistical Analysis

Statistical analysis details are provided in the online **Supplemental Material**.

Results

SARS-CoV-2 antibody avidity analytical validation

Determination of concentration independent range in clinical specimens

As described in the assay description, accurate antibody dR requires only sufficient amounts of antibody to be loaded onto the probe surface. To evaluate and minimize this potential source of artifact associated with these label-free methods, dilution studies were performed using four specimens with high TAb measurements (in the range 1171-10872 RFU) and plotted against the dR. (**Figure 2A**). Based on these studies, it was determined that the concentration-independent range for this assay was between 20 and 615 TAb RFU. (**Figure 2B**) Therefore, any specimen with a TAb greater than 615 RFU was first diluted into the 20-615 RFU range prior to determining the dR.

Sars-CoV-2 antibody avidity assay characterization with model COVID-19 purified antibodies

The dissociation profiles of five different antibodies over time are demonstrated in **Figure 3A**. Antibodies of varying RBD binding strength (online **Supplemental Table 2** displayed different dRs and therefore, different dissociation profiles. The dRs were measured at proper antibody loading concentrations (0.06-30ug/ml, varying for different antibody) and the avidity measurement was found to be concentration (and therefore fluorescent signal) independent, as long as the initial fluorescent signal (Signal_0) is in the proper range (**Figure 3B**).

Precision and Interference

The imprecision was determined by running the high and low level of pooled patient samples (n=5-10) five times per day on five different days. The imprecision of the TOP avidity assay was

7.5% and 9.8% at two dR levels of 7.23×10^{-4} 1/s and 4.66×10^{-4} 1/s, respectively. The stability of samples at 2-4°C refrigerated conditions was at least 5 days (variation: < 8%).

The TOP-Plus avidity assay was tested with common endogenous immunoassay interferences. Avidity of two SARS-CoV-2 model purified antibodies first was measured in pooled SARS-CoV-2 negative serum, followed with spiking either biotin, bilirubin, hemoglobin or triglyceride and measuring antibody avidity in presence of each potential interferents. The TOP-Plus avidity assay displayed no interference from the listed components up to the tested concentrations.

(online **Supplemental Table 3**)

No cross-reactivity was displayed in sera from HIV, EBV and Rheumatoid factor positive patients. All six samples were negative (online **Supplemental Table 4**) for TAb. Potential heterophilic antibody interference was further evaluated by performing spike-in experiments with a pooled HAMA sample, which had an RFU of 4 (cutoff RFU: 20), indicating no TAb assay interference by HAMA. There was no significant difference in values between the HAMA diluent and those by negative serum diluent, indicating that HAMA would not interfere with TAb and hence avidity measurements. (online **Supplemental Table 5**)

Correlation of TOP-Plus avidity assay to BLI

BLI is a well-established technique in avidity measurement (25, 26). To further validate the performance of TOP-Plus' avidity assay, Gator was used as a BLI reference method to measure the avidity of 12 purified COVID-19 antibodies. These values were compared to the avidity

measured by the TOP-Plus avidity assay. It was found that the TOP-Plus avidity assay measurements correlated well ($r=0.88$) with the Gator measurements. **(Figure 3C)**

Evaluation of clinical utility

TAbs versus antibody avidity levels based on severity and persistence of symptoms

It had been previously shown that TAb levels were significantly lower in the outpatient compared to inpatient populations.(23) For a better understanding on whether there was a similar relationship between TAb levels and severity of illness in this convalescent cohort, the individuals TAb levels were stratified by the severity of acute infection, as assessed by the World Health Organization's (WHO) *Ordinal Clinical Progression/Improvement Scale*.(27) (online **Supplemental Table 6**) TAb were confirmed to be higher in COVID-19 individuals who had limitations in their activities due to COVID-19 symptoms (WHO Ordinal Scale 2) and still higher in hospitalized patients (WHO Ordinal Scale ≥ 3). This was observed early in convalescence at 1.3 months and continued to 6.2 months post-infection. **(Figure 4A).**

Study participants had been asked about symptom persistence at their 6 month follow up visit and were stratified retrospectively based on the responses.(22) Persistent symptoms included fatigue, dyspnea, athletic deficit, or ≥ 3 solicited symptoms beyond 6 weeks of symptom onset. TAb levels were elevated in individuals who displayed persistence of symptoms beyond 6 weeks of symptom onset, when compared to those with no persistence of symptoms. **(Figure 4B)** Unlike TAb, antibody avidity remained unchanged across all WHO Ordinal Scales or in those with persistence of COVID-19 symptoms. **(Figure 4C and 4D).**

SARS-CoV-2 antibody dynamics during early convalescence

TAb was evaluated in 80 individuals with confirmed or suspected COVID-19 (21, 22) ~1.3 months and again ~6.2 months after the time the SARS-CoV-2 infection was first confirmed or suspected. TAb decreased over time in 58 out of the 80 individuals (**Figure 5C**). The median TAb at 1.3 months was 525 RFU (IQR 165.5-1943) compared to 380.5 RFU at 6.2 months (IQR 136-1103) ($P=0.0042$) (**Figure 5A and 5C**). The decrease in TAb in this cohort mirrored the decrease in IgG and IgM levels that were measured using the same TOP biosensor (previously described in prior publications). (22, 24)

SNAb had been previously shown (23) to correlate well with both the plaque reduction neutralization test (PRNT) and the pseudo virus neutralization test (PsV), two well-established SARS-CoV-2 virus neutralization tests. In the SNAb assay, the percentage of RBD-ACE2 binding is defined as $\%B/B_0 = [\text{sample relative fluorescence unit (RFU)}/\text{negative control RFU}] * 100\%$. This current study found that the neutralization activity decreased over time in 69 out of the 80 individuals (**Figure 5F**), as determined by the SNAb assay. The median % ACE binding at 1.3 months was 42.32 %B/B₀ (IQR 14.14-67.00) compared to 65.55 %B/B₀ (IQR 38.85-89.71) ($P < 0.0001$; **Figure 5D-E**). Together with the decrease in TAb, these results are indicative of not only overall SARS-CoV-S antibody levels diminishing over time, but also a diminishment in the total antibody neutralization activity.

In contrast, the antibody avidity increased in 76 out of 80 individuals over this same time period (**Figure 5I**), as indicated by a significant decrease in the median dR: $9.685 \times 10^{-4}/s$ at 1.3 months post-infection to $5.830 \times 10^{-4}/s$ at 6.2 months post-infection ($P < 0.0001$; **Figure 5G and 4H**).

This reflected a median increase of $3.855 \times 10^{-4}/s$ or 39.8%. (**Figure 5H**).

Comparison of SARS-CoV-2 antibody avidity post-vaccination to that of early convalescence

To demonstrate the avidity assay's potential clinical utility in evaluating the antibody response to SARS-CoV-2 vaccination, antibody avidity was measured in individuals who were vaccinated with the mRNA-1273 vaccine circa 1 month (25-28 days post-vaccination, median 28 days) after their first dose of the vaccine. These were compared to the 20 individuals in the previously described COVID-19 positive cohort that had specimen collected circa 1 month post-infection (21-30 days post symptom onset, median 26 days). The antibody avidity levels in vaccinated individuals did not significantly vary from COVID-19 individuals circa 1 month post exposure. (**Figure 6**)

Discussion

Antibody avidity testing is not a new concept in the evaluation of an antibody response to infection or vaccination. Typically, antibodies generated early in a primary infection bind weakly to their respective antigen and exhibit low avidity or functional affinity. (28) However, overall avidity towards an antigen(s) increases as the response matures through somatic hypermutation particularly of the variable loops of antigen-binding sites of B cell receptors and selective survival in the germinal center. (29, 30) As antibody avidity typically increases over time and is an indicator of a more mature antibody response, antibody avidity could be applied in assessing the efficacy of COVID-19 vaccination, immunity to SARS-CoV-2 and screening donors for convalescent plasma antibody therapies.

Studies have tried to explain the SARS CoV-2 antibody response variability by focusing on antibody avidity.(31) This study monitored avidity by measuring the relative dissociation rate of SARS-CoV-2 specific antibodies from RBD and compared it to TAb and SNAbs, allowing for the assessment of the antibodies' strength in binding to the virus. The dR inversely associates with the average antibody's residence time at the epitope. Antibodies with lower dR values tightly bind to RBD and therefore may be more efficient in clearing the virus and neutralizing infectivity, i.e., blocking the entry into target cells.(32)

Antibody affinity reflects the rate constants of association and dissociation of an antibody with its target antigen ($K_D (M) = k_{off} (1/s) / k_{on} (1/Ms)$). In many serological applications, measurement of antibody-antigen interactions becomes a complicated process. Therefore, the most common approach is to disrupt the antibody-antigen binding by chaotropic agents (such as urea). The avidity is then assessed by measuring the change in the degree of release of antibody from the antigen by the chaotropic agent.(17, 19) As a result, the assessed avidity of antibody depends on its resistance to the chaotropic agent and may not truly represent the avidity of antibody toward the antigen.(13)

The TOP-Plus avidity assay presented here measures the relative rate of dissociation of SARS-CoV-2 antibodies from the RBD antigen in plasma. However, this assay distinguishes itself from others in that it does not apply a chaotropic reagent. Therefore, the measured dR values better reflect the natural relative dissociation rate of antibodies from their target antigen than the conventional approaches where chaotropes may alter the native structure of the antigen or antibody. (13)

Previously no one assay could evaluate total antibody levels, individual IgM and individual IgG levels as well as avidity. The new TOP-Plus biosensor panel comprises five assays, allowing for TAb, SNAbs, IgG and IgM levels as well as avidity testing on the same platform using the same biosensor principles with specific application applied for each assay. This probe was able to assess the overall decreasing trend in TAb and SNAbs (**Figure 5**), in addition to the previously reported decreases in IgG and IgM.(22)

Our findings of the decay in total SARS-CoV-2 antibodies and neutralization antibody activities are consistent with the previous studies.(10, 16, 21, 33-37) However, SARS-CoV-2 antibody avidity did not show the same pattern of diminishment during the first six months of infection (**Figure 5**). Our observation is congruent with the previous report (22) that memory B cell responses continue to evolve and express antibodies with increased neutralizing potency and breadth. Therefore, the increased antibody avidity is indicative of continued evolution of the humoral response.

With the ongoing world-wide SARS-CoV-2 vaccination programs, such a panel could play a major role in monitoring the vaccination response in individuals, but also on a larger epidemiological scale. As there are multiple dimensions in evaluating the humoral immune response to the SARS-CoV-2 vaccine, TOP-Plus has potential for monitoring adequate humoral immune response to the SARS-CoV-2 as a whole. Monitoring only for overall SARS-CoV-2 antibody levels or neutralization antibody levels could create a false impression of a diminishing immune response while the TOP-Plus with its avidity assay may assure an appropriate immune response maturation. Indeed, our initial studies show that at least in the early weeks post-

vaccination, vaccinated individuals display similar antibody avidity when compared to those in a comparable period post-infection and it is hypothesized that the antibody avidity 6 months post-vaccination will strengthen as has been demonstrated post SARS-CoV-2 infection. (**Figure 6**) However, future studies would need to longitudinally follow vaccinated individuals to fully validate the assay for this purpose.

Different SARS-CoV-2 mutations raise concerns of the emergence of a more contagious or virulent variant. Therefore, there is a need for the development of assays that can properly characterize the humoral immune response to the SARS-CoV-2 vaccine and evaluate for immunity against these emerging virus variants. Although the current iteration of the TOP-Plus avidity assay measures the antibody avidity against the initially described SARS-CoV-2 RBD, the capability in measuring antibody avidity against other virus variants could be extended by replacing the RBD reagent of the assay (and probe) to the corresponding RBD of other virus variants.

The fact that only convalescent serum specimens were evaluated in this study is a limitation. The presented data cannot speak to the avidity maturation during the acute phase of infection and will require further studies to determine the full utility of TOP-Plus' avidity assay in acutely ill patients. As it is a newly evolved virus, it would be expected that the antibody avidity for SARS-CoV-2 antigens during primary infection would be weak and this avidity would increase over time. However, during the acute stages of infection, IgM could precede the IgG response and it could be postulated that the overall avidity may display an initial spike during the acute stage of

infection due to the multimeric structure of the IgM antibody, masking the primary infection's expected weaker avidity.

In conclusion, this TOP-Plus biosensor panel is a versatile sensing platform with high precision and an ability to measure SARS-CoV-2 TAb, SNAbs, individual IgG and IgM antibody levels along with the antibody's long-term avidity. This combination of all-in-one testing will be a valuable asset in monitoring not only convalescent COVID-19 patients, but also the status of individuals' COVID-19 vaccination response.

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J. Yee and A. Sukhu performed the experiments. Y. Hao and S. Rand helped collect data. S.E. Racine-Brzostek wrote the manuscript, performed analysis, generated figures and aided in the review of the investigational findings. Z. Zhao oversaw the project, including conceptualization, interpretation of the data, statistical analysis and writing of the manuscript. H.S. Yang helped with editing the manuscript and interpretation of the data. A. Chadburn and M.M. Cushing helped review the manuscript. C. Gaebler collected specimens, analyze the patient data and review of the manuscript. R. Zuk oversaw the methodology for the TOP-Plus method development and conceptualization of the project. M. Karbaschi performed formal analysis for the TOP assay development, provided analytical data analysis and helped edit the manuscript. P.J. Klasse helped with editing the manuscript and performed a formal review. M. Caskey collected specimens, analyze the patient data and review of the manuscript. M.C. Nussenzweig collected specimens, analyze the patient data and review of the manuscript. Y. Shi helped with conceptualization of the project and design the experiments.

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Figure Legends

Figure 1

Sars-CoV-2 antibody avidity assay principle. An RBD precoated probe is sequentially incubated in microwells containing 1) sample to capture SARS-CoV-2-specific antibodies 2) wash buffer 3) detection biotinylated RBD 4) wash buffer 5) Streptavidin-Cy5 conjugate. The fluorescent signal is then measured (Signal₀). After that, probe with the immobilized immunocomplex goes into repetitive dissociation cycles by multiple incubations in PBST as dissociation buffer. After each incubation, fluorescent signal is measured (Signal_t). At the end of measurement, dissociation curve is constructed by plotting the normalized fluorescent signal (Signal₀/Signal_t) over time. The dR is then calculated by fitting a first order reaction kinetics to the dissociation curve.

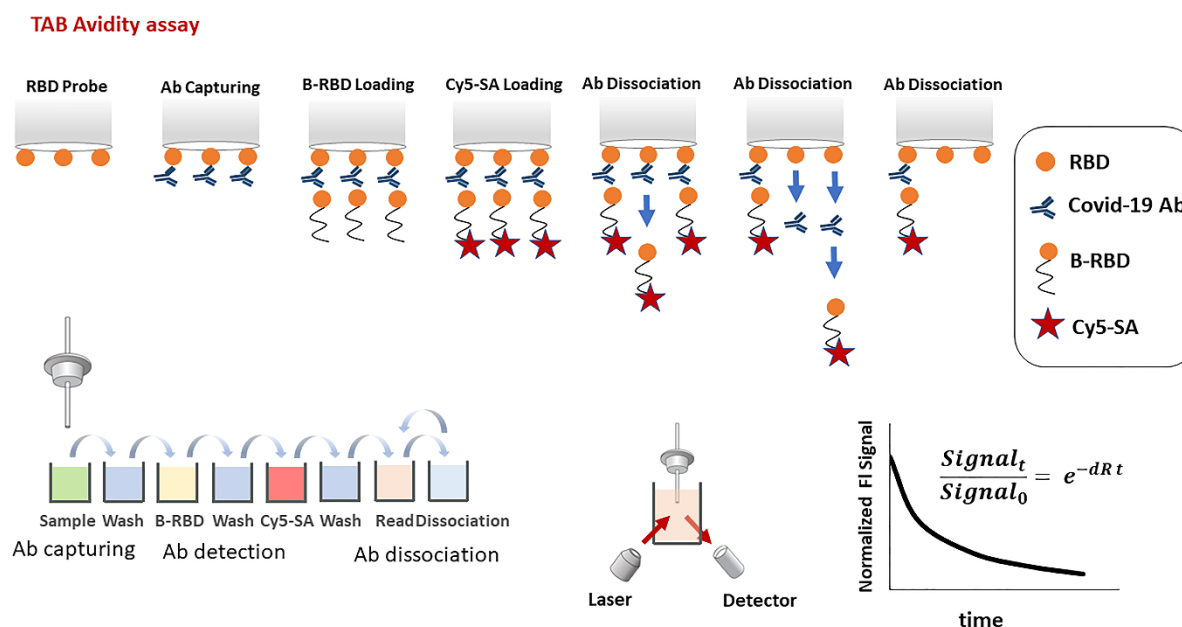


Figure 2

Determination of concentration independent range. A) Dilution studies were performed using four randomly selected specimen with high TAb measurements, (1171-10872 undiluted RFU) and plotted against the disassociation rate. B) The dissociation rate was independent of TAb concentration between 20 and 615 TAb RFU.

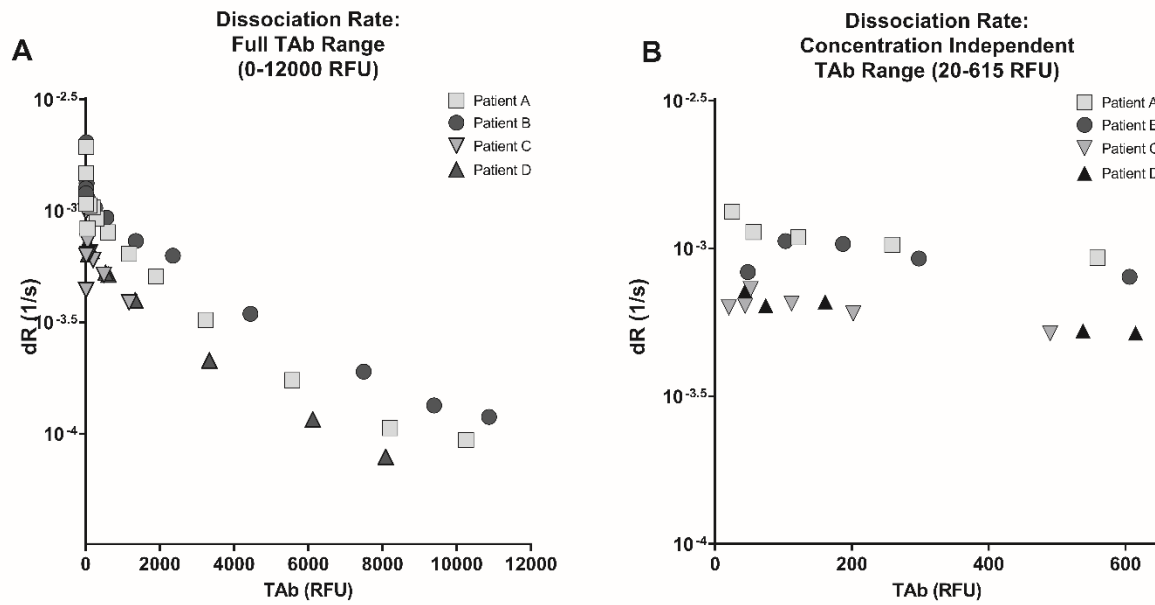


Figure 3

Sars-CoV-2 antibody avidity assay characterization with model COVID-19 purified antibodies.

A) Dissociation curve measurement of COVID-19 negative human serum spiked with five different COVID-19 antibodies. Signal₀/Signal_t is the normalized fluorescent signal on the y-axis. B) Relative dissociation rate measurement at varying levels of Signal₀. The COVID-19 negative human serum was spiked with seven COVID-19 antibodies at different levels and measured. C) Correlation between the SARS-CoV-2 avidity assay (dissociation rate measurement) and the Bio-Layer Interferometry (BLI) measurement (k_{off}). Correlation between the two assays were assessed by Spearman correlation coefficient.

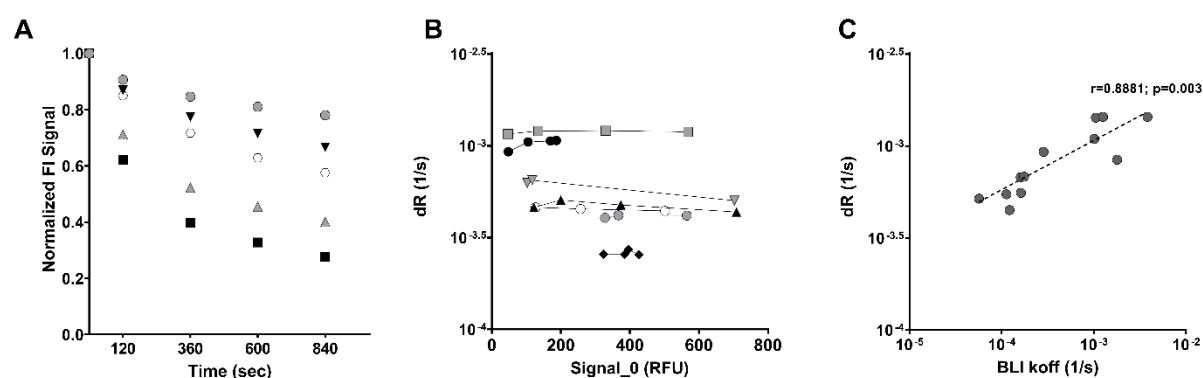


Figure 4

Antibody avidity is independent of prior COVID-19 severity (as determined by the WHO Ordinal Scale for Clinical Improvement) or persistence of symptoms. (A) TAb levels were higher in COVID-19 individuals who had limitations in their activities due to COVID-19 symptoms (WHO Ordinal Scale 2) and higher still in hospitalized patients (WHO Ordinal Scale ≥ 3) at 1.3 and 6.2 months post-infection. (B) At 1.3 and 6.2 months post-infection, TAb levels were elevated in individuals who displayed persistence of symptoms beyond 6 weeks of

symptom onset when compared to those without persistence of symptoms. In contrast antibody avidity remained unchanged across (C) all WHO Ordinal Scales or (D) with persistence of COVID-19 symptoms. Note: Persistent symptoms included fatigue, dyspnea, athletic deficit, or ≥ 3 solicited symptoms beyond 6 weeks of symptom onset.

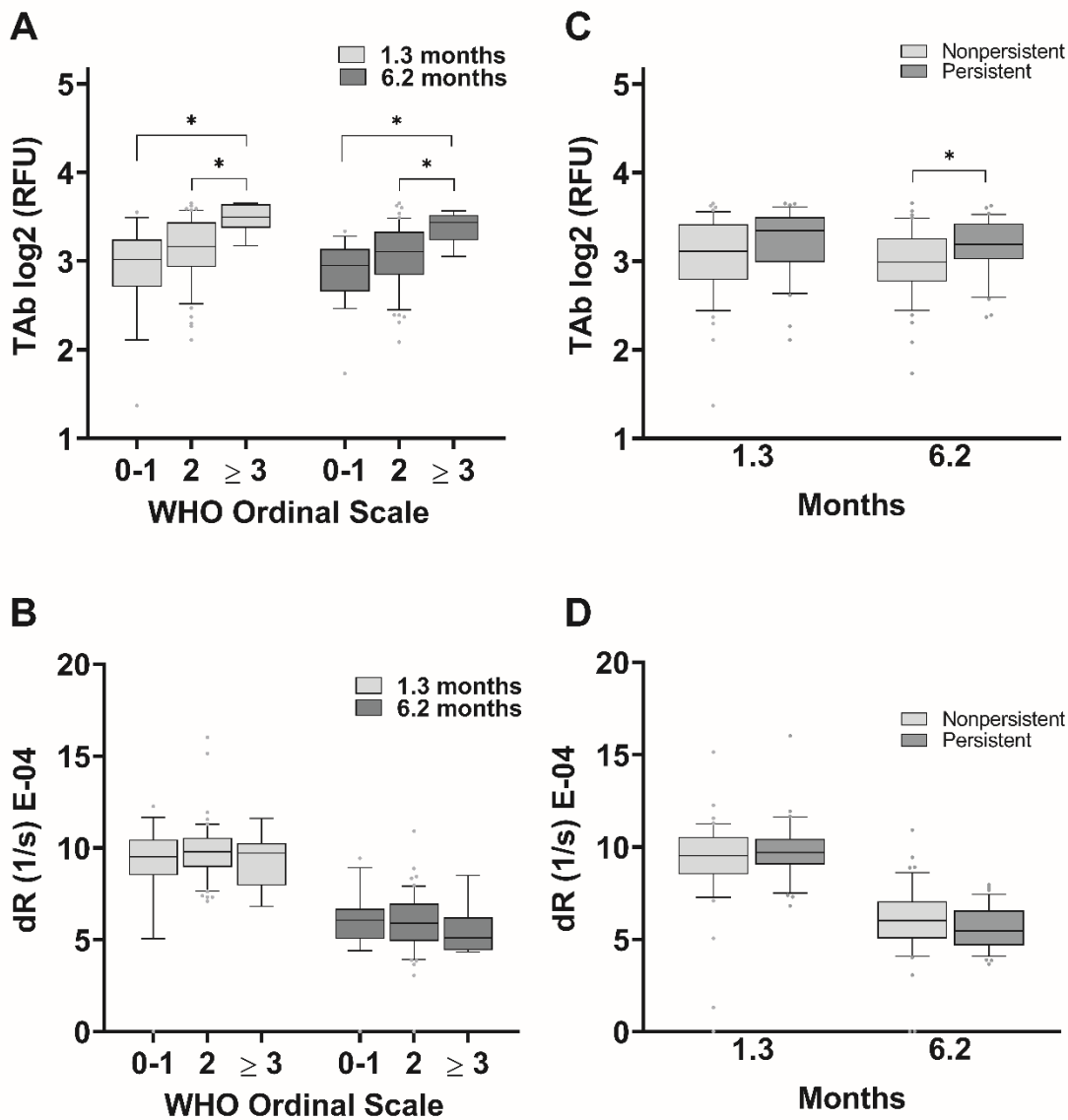


Figure 5

SARS-CoV-2 antibody dynamics during early convalescence.

Eighty COVID-19 positive individuals' TAB, SNAb and antibody avidity levels were plotted at 1.3 and 6.2 months (A,D,G). The change in TAB, SNAb and avidity over time (1.3 to 6.2 months) is displayed in panels B, E, H. Individual data pairs for TAB, SNAb and avidity are plotted in Panels C, F and I. TAB and SNAb activities (inversely associates with %B/B0) in majority of individuals decreased over time. In contrast, the avidity (inversely associates with dR) increased during this time. Note: The SNAb assay read-out is the percentage of RBD-ACE2 binding (%B/B0), which inversely correlates with the SNAb activity. The avidity assay measures the dissociation rate of SARS-CoV-2 antibodies from the RBD, which is inversely correlated with the antibody avidity.

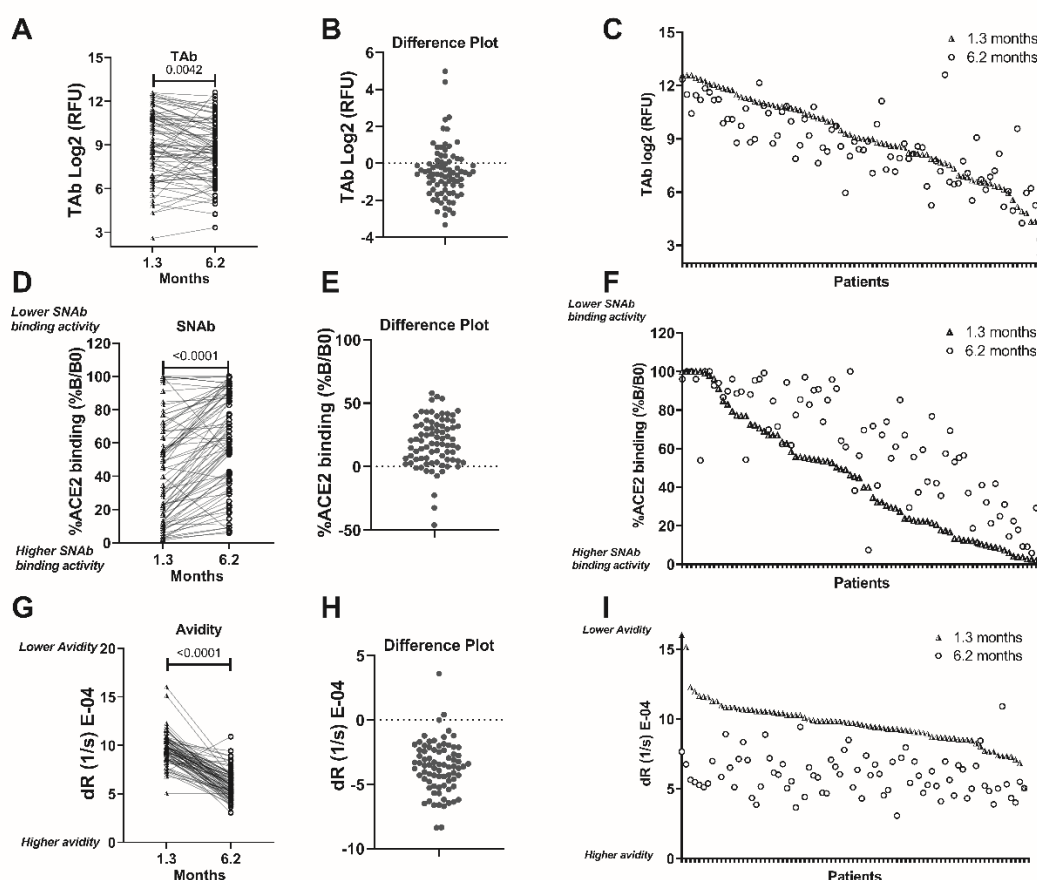


Figure 6

Comparison of antibody avidity in individuals previously infected compared to those who were vaccinated with the mRNA-1273 vaccine. Sera of 10 individuals, approximately 1 month (25-28 days post-vaccination, median 28 days) after their first dose of the mRNA-1273 vaccine, were analyzed for antibody avidity. These were compared to the 20 individuals in the previously described COVID-19 positive cohort that had specimen collected approximately 1 month post-infection (21-30 days post symptom onset, median 26 days). For further comparison, the antibody avidity at approximately 6 months post-infection in these 20 COVID-19 positive were also displayed (165-204 days post-vaccination, median 183 days).

