ID# BIORXIV/2022/487697

Epitope Mapping of SARS-CoV-2 Spike Protein Reveals Distinguishable Antibody Binding Activity of Vaccinated and Infected Individuals

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ABSTRACT

A multitude of studies have attempted to characterize the antibody response of individuals to the SARS-CoV-2 virus on a linear peptide level by utilizing peptide microarrays. These studies have helped to identify epitopes that have potential to be used for diagnostic tests to identify infected individuals, however the immunological responses of individuals who have received the currently available mRNA vaccines have not been characterized. We aimed to identify linear peptides of the SARS-CoV-2 spike protein that elicited high IgA or IgG binding activity and compare the immunoreactivity of infected individuals to those who received recommended doses of either the Moderna mRNA-1273 or Pfizer BNT162b2 vaccines by utilizing peptide microarrays. Our results revealed peptide epitopes of significant IgG binding among recently infected individuals, many of which are located near functional domains implicated in the high infectivity of SARS-CoV-2. Vaccinated individuals were found to have less intense antibody binding activity than those acutely infected, yet novel markers of IgG binding were identified in the vaccinated group.

INTRODUCTION

Emerging variants of SARS-CoV-2 continue to make up an increased proportion of reported cases (1). These variants contain mutations that may play a role in viral infectivity and decrease effectiveness of the current vaccines (2, 3), increasing the importance of epitope-based

studies of the antibody response of vaccinated individuals and previously infected individuals. Studying epitopes at the peptide level helps to reveal which mutations in currently prevalent variants may increase infectivity and transmissibility affected by alteration of these changes in the viral proteins. In addition, epitopes that are found to have consistent antibody binding may serve as a useful diagnostic target to assess previous infection or general protective status of individuals. Viral load can reach undetectable levels within 1-2 weeks after symptom onset (44) and antigen tests are sometimes plagued by low sensitivity (39). Tests that help gain an understanding of the epitopes that elicit significant antibody binding may prove exceptionally useful for diagnosis and vaccine development. Epitopes found to be common targets of antibodies could be incorporated into diagnostic tests.

The peptide microarray is a tool with many immunological applications, including the screening of antibody binding activity against antigens or entire pathogenic proteomes. This information can provide answers regarding the presence of potential epitopes and a characterization of the target pathogen's mechanisms of infection. As entire targeted viral proteomes can be bound to microarrays, the process of screening for antibody binding is both fast and reproducible. These capabilities, alongside easy to manage data outputs make the peptide microarray a particularly powerful tool to perform systematic epitope profiling of viral pathogens such as SARS-CoV-2.

Previous work utilizing the peptide microarray have focused heavily on mapping the humoral responses to the SARS-CoV-2 proteome using samples from individuals currently or previously infected with SARS-CoV-2 (5, 7–11). SARS-CoV-2 epitopes that elicit a significant IgG and/or IgM antibody response in infected or previously infected samples have been identified on the spike (S), nucleocapsid (N), membrane (M), Orf1ab, and Orf3a proteins. While cross-reactivity with other common coronaviruses has been observed, (5,7) there are many epitopes which demonstrate highly specific reactivity with SARS-CoV-2 antibodies distributed across the SARS-CoV-2 proteome. A multitude of epitopes have been identified in numerous previous studies (3, 5, 7–9, 11), both in commercially available and independently designed peptide microarray slides. These findings support using a peptide microarray approach to locate highly specific and sensitive SARS-CoV-2 human B Cell epitopes.

Several studies (5, 7–11) have demonstrated that peptide microarrays can discriminate between mild symptomatic, severe symptomatic, and eventually fatal cases of SARS-CoV-2 based on the antibody reactivity of specific SARS-CoV-2 epitopes (8, 11). Additional microarray studies have also demonstrated that specific epitopes identified using a peptide-microarray can elicit a strong neutralizing antibody response (10). These findings indicate that there may exist a linear epitope or group of linear epitopes that can serve as a marker of the protective status of individuals to SARS-CoV-2 infection. While this is an exciting prospect, previous microarray epitope mapping studies have been focused exclusively on the comparison of infected and naive individuals. To our knowledge no study has utilized a peptide-microarray approach to study individuals who received the currently available mRNA vaccines, Moderna mRNA-1273 and Pfizer BNT162b2. It is to be expected that a peptide microarray is capable of differentiating the

immune response of vaccinated individuals by analyzing the antibody binding to the targeted SARS-CoV-2 spike protein peptides.

Herein we report a study of IgG and IgA antibody reactivity of individuals vaccinated with the Pfizer and Moderna mRNA vaccines against linear peptides. As the Moderna and Pfizer vaccines contain the sequences for the SARS-CoV-2 spike protein, our study has focused mainly on the spike protein region. Data herein identifies epitopes that have the potential to serve as markers to assess SARS-CoV-2 protection in addition to gaining a general characterization of IgG/IgA antibody response against SARS-CoV-2 in vaccinated individuals. These epitopes may be useful in the study of immune response development in vaccinated individuals as well as aid in the development of immunity or detection assays with broad potential uses such as evaluating vaccine efficacy against variant strains of SARS-CoV-2.

MATERIALS AND METHODS

1.1 Infected, Vaccinated Serum & Control serum samples

Serum samples were purchased from Raybiotech (Atlanta, GA) and Reprocell (Beltsville, MD). Samples were divided into four groups:

- SARS-Cov-2 positive individuals (n=30)
- Individuals who received either the Moderna or Pfizer mRNA vaccines (n=17)
- A naive negative control group (n=10)

Among the 10 negative controls, five serum samples were collected prior to the pandemic before the end of 2019 (n=5). There are five paired vaccinated individuals whose serum samples were collected 1-3 days before receiving the first dose of either the Pfizer (3 paired) or Moderna (2 paired) vaccine. These five were self-reported as never infected before their vaccination. The post-vaccinated sera were collected between 6-44 days after receiving their second dose of the Moderna or Pfizer mRNA vaccine. Basic demographic information for infected patients can be found in Table S1 and subject symptom information is available in Supplemental Table S3. Patient information for vaccinated and negative subjects can be found in Supplemental Table S2. All purchased infected sera was inactivated with a 4.0% Triton X-100 treatment prior to their arrival at our facility. All samples were immediately aliquoted and stored at -80° C after receiving them.

1.2 Peptide Microarrays

For our systematic proteome analysis, PEPperCHIP® (Heidelberg, Germany) SARS-CoV-2 Proteome Microarray slides were used for all samples. These slides contain a series of linear peptide sequences that cover the entire SARS-COV-2 proteome. Each of these peptides are printed in duplicate and consist of 15 amino acids in total, 13 of which overlap with neighboring SARS-CoV-2 peptide sequences. Additionally, the slides contain certain mutated peptide

sequences found in SARS-CoV-2 variants and a set of influenza and polio peptides which were utilized as positive controls.

Prior to performing the procedure, solutions of PBST wash buffer (Phosphate-buffered saline with 0.05% Tween 20, at 7.4 pH), and pH 7.4 1mM Tris dipping buffer were prepared. All solutions were filtered using a 0.44-micron vacuum filter kit and pH was adjusted to 7.4 with the addition of 3M HCl if necessary. Microarray slides were initially incubated in a solution of 0.05% Tween PBS (7.4 pH) and 10% Blocking Buffer (Rockland Blocking Buffer for 15 minutes at 23°C). Microarray slides were aspirated and then blocked with Rockland Blocking Buffer for 30 minutes at 23°C. After blocking, slides were again aspirated and probed with a mixture of fluorescent secondary antibodies that would be used for peptide binding detection consisting of Rabbit Anti-Human IgG DyLight 800 (Rockland Immunochemicals), Alexa Conjugated Goat Anti-Human IgA 680 (Jackson ImmunoResearch), and Mouse Anti-HA Dylight 680 (PEPperCHIP, Heidelberg, Germany). This prescreen of secondary antibody treatment was used to reveal any non-specific secondary antibody interactions with the slide. After performing 3 PBST washes of one minute each, slides were treated with sera diluted 1:500 in a 10% blocking buffer/ PBST solution and incubated overnight at 4°C. Following this overnight incubation slides were washed 3 times with PBST for one minute each and dipped into the previously prepared 1mM Tris Solution. Detection of IgG was performed via treatment with Rabbit Anti-Human IgG DyLight 800 (Rockland Immunochemicals) for 45 minutes at 23°C. After performing 3 PBST washes of one minute each, slides were then treated a second time with samples diluted 1:200 in 10% blocking buffer/ PBST and incubated overnight at 4□. After the second overnight incubation, samples were washed 3 times with PBST wash solution (1 minute each) and treated with Alexa Conjugated Goat Anti-Human IgA 680 (Jackson ImmunoResearch) for 45 minutes at 23°C for detection of IgA. Before scanning, a final set of 3 washes of 1 minute each using PBST wash buffer solution was performed followed by dipping the slides 3 times in the 1mM Tris Solution.

Fluorescent signals were acquired using an Innopsys Innoscan 710-IR Microarray scanner (4). Slides were scanned at wavelengths of 670 nm for IgA detection and 785 nm for IgG detection and at a resolution of 30uM for both wavelengths. Fluorescent values were retrieved using Innopsys Mapix Microarray Analysis Software.

1.3 Epitope Validation

Prior to data analysis of serum-treated slides, the tiff image files acquired from the probing of slides with the secondary antibody solution were analyzed for any slide artifacts (11, 12). Artifact sequences from the prescreen of secondary antibody treatment were excluded from further data analysis. Any microarray slides that did display significant reactivity with polio and HA influenza positive control peptide spots were considered invalid.

For data analysis, we used the background-subtracted median intensity values acquired from the Innopsys Mapix Microarray Analysis Software. To identify potential epitopes, we first averaged these median fluorescent intensity values of each peptide found within the spike protein

for each experimental group. We then selected peptides in which the average log_2-normalized median intensity value of the infected group was at least 1.5-fold greater and had p-values of < 0.05 from an unpaired t-test comparison to the average log_2-normalized median intensity value of the negative controls. Among these selected peptides we compared the average log_2-normalized median intensity value of each peptide to their neighboring peptides. Peptides that had significantly different fluorescent intensity values were screened out, and the remaining peptides were recognized as potential epitopes (5, 13)

1.4 ELISA Testing

In-house ELISA tests were utilized to detect levels of IgA and IgG antibodies against SARS-CoV-2 receptor-binding domain (RBD) antigen. Samples were diluted at 1:10,000 in a 1% milk PBST solution. 100uL of serum samples (in duplicate) were added to ELISA plates that were coated with 150ng of SARS-CoV-2 RBD antigen per well. The plates were incubated overnight at 4^oC and washed three times with a 1% Tween20 PBS solution. Secondary detection antibody solutions of HRP conjugated polyclonal mouse anti-human IgG antibodies (SouthernBiotech) diluted 1:8000 in 1% milk PBST and HRP conjugated monoclonal mouse anti-human IgA1/IgA2 antibodies (SouthernBiotech) diluted 1:4000 in 1% milk PBST were used to detect IgG and IgA respectively. Secondary antibody solutions were incubated at room temperature for 1 hour on an orbital shaker set at 200 rpm. Plates were washed three times with a 1% Tween 20 PBS solution and developed for 10-minutes with a TMB substrate solution. 3M HCl was added and plates were scanned using the SpectraMax ID3 Plate Reader (Molecular Device, San Jose, CA). (Table S4)

RESULTS

2.1 SARS-CoV-2 Infected Individuals Exhibited the Highest IgG Binding Closer to Date of Symptom Onset

To identify reactive epitopes, we looked at peptides in which the average log_2-normalized intensity value of either of the two infected sample groups had a fold-change value of at least 1.5 and yielded adjusted p-values of < 0.05 from an unpaired t-test when compared to the mean log_2-normalized intensity value from the negative control group (11, 13). Using these criteria, nine peptides with significantly higher IgG antibody binding were identified in the spike protein region (Figure 1a and Table 1) among infected individuals.

Using the same approach of epitope identification with our vaccinated groups, no peptides were found to meet the criteria for significant IgG binding activity. Two peptides were found to have p-values less than 0.05, but these peptides did not have fold change of at least 1.5-folds (Fig. 1b). Of the nine peptides identified as significant in the infected sample group, five were also found to have significantly greater IgG binding than the vaccine group. The two peptides with no significant differences in binding activity between the recently infected group and vaccinated group were peptides S_1141 and S_1247. The SARS-CoV-2 RBD IgG titer of the vaccinated individuals was comparable to the IgG titers of infected samples according to

information provided by the sample provider, Raybiotech and our own in house RBD ELISA testing (Table S4).

Peptides found to have significant IgG binding activity from the recently infected (<3 weeks post symptom onset) group that are located on the spike protein appear to be distributed throughout the protein (Figure. 2). Identified reactive epitopes that meet the criteria as described above included a one located within the RBD (S_0343, S_0345), one located the putative fusion peptide domain (S_0671), one found in proximity to the identified Heptad Repeat (HR) 2 sequence (S1141), and a Cys-rich sequence (S_1247, S_1249, S_1251) neighboring what is thought to be a transmembrane domain (43) located in the C terminal of S2 subunit. Among these peptides, those that were found to have relatively higher intensity amongst the vaccinated group (S_1159 and S_1247) were both found in the S2 region. Five mutations common in current common variants were identified within at least one peptide sequence (Table 3). The mutations present in our identified peptide epitopes were also found to be well distributed among these common variants.

2.2 No Significant Difference in IgG Epitope Profile of Spike Protein Between Individuals Who Received Moderna Vaccine and Those Who Received Pfizer Vaccine

While no significant antibody binding was detected within our vaccinated sample pool, we decided to compare the IgG binding activity of individuals who received the Moderna (n=8) and Pfizer mRNA vaccines (n=9). Comparison of the Moderna and Pfizer vaccinated sample groups revealed little in terms of significant differences in IgG epitope binding activity in samples between the two vaccines. Both vaccinated groups appeared to have more IgG binding activity to epitopes in the S2 region of the spike protein (Figure 3), although peptide S_0343 located in the RBD region appeared to have significant binding in certain individuals of both groups. To compare the peptides that elicited the highest IgG binding in both groups, peptides that displayed fluorescent intensity values that were 3.0 standard deviations above the mean in each group were identified (Table 2). 11 peptides were found to have intensities above this cutoff in the Moderna sample group and two peptides were identified as being above this cutoff in the Pfizer sample pool. Both peptides above 3.0 deviations in the Pfizer vaccinated group also met this criteria in the Moderna vaccinated group. Of these 11 peptides, six were either identified as having significant IgG binding activity in the recently infected sample group, or overlapped with a peptide found to have significant IgG binding activity. None of the peptides in either of these groups met our criteria for significantly high IgG binding, so the findings of this comparison are limited.

2.3 No significant IgA Binding Activity to SARS-CoV-2 Spike Protein Epitopes Identified in Infected and Vaccinated Individuals

To investigate potential IgA epitopes of the spike protein we used the same approach as we used to identify IgG epitopes. No significant IgA epitopes were identified in either of the infected groups or the vaccinated group. The IgA titer information of the samples provided by Raybiotech was limited, but in-house ELISA testing of the RBD antigen revealed low levels of

IgA binding throughout both infected and vaccinated groups (Table S4). As the microarrays we are utilizing contain the entire proteome of SARS-CoV-2, we were able to identify significant IgA peptide binding outside of the spike protein. As the scope of this report is solely focused on the spike protein these epitopes have been excluded.

DISCUSSION

In order to assess the potential of incorporating the discovered peptides into a viable therapeutics or diagnostics, we investigated the conservation of sequences among other closely related betacoronaviruses and the proximity of the sequences to mutations found in current dominant variants as compared to the Wuhan strain. Previous studies have established that antibodies that bind to certain linear epitopes found in the SARS-CoV-2 proteome are found to be reactive to conserved peptide stretches in other coronaviruses (CoVs) (12). Aligning the epitopes to closely related CoV helped to assess the potential for nonspecific antibody binding by antibodies present due to exposure to other betacoronaviruses. In addition, with the current global prevalence of variant strains such as the Delta, South African, and recently emerged Omicron variants, it is important to investigate whether the peptide sequences contained or were in proximity to any mutations commonly found in the variants.

3.1 Peptide Sequence Alignment Analysis

Most human CoVs cause relatively mild upper respiratory tract infection (common cold), such as HCoV-229E, HCoV-NL63, belonging to the alphacoronavirus genus. The SARS and MERS CoV viruses belong to the betacoronavirus genus (lineage B). They are associated with severe lower respiratory tract infections and are considered to be major public health threats. In order to predict potential antibodies that may exhibit cross-reactive binding with peptides of other CoVs, we created alignments of the spike protein sequences found in the seven known human infecting coronaviruses (hCoVs) (Figure. 4). The alignments revealed that the identified peptides were poorly conserved outside of the lineage B hCoVs. Of the nine peptides identified amongst the acutely infected group, the peptides S_1141 and S_1247-S_1251 were found to be relatively well conserved amongst the human infecting coronaviruses. Peptide S_1141, located in proximity to the HR2 (heptad repeat domain 2) sequence was found to have relatively similar identity, with 6/15 amino acid residues being shared amongst all of the peptides and 4 amino acid residues being classified conserved or semi-conserved mutations. The sequence in proximity to S_1141 has also been identified as being potentially cross reactive in previous microarray studies of SARS-CoV-2 (7). The other identified peptides that are relatively well conserved are S_1247-S_1251. Located near the C-terminus of the spike protein just outside the transmembrane domain, these contiguous peptides contain highly conserved cysteine residues which are thought to be imperative for membrane fusion **(37)**.

3.2 Peptide mutations found in Common SARS-CoV-2 Variants

With the current prevalence of certain SARS-CoV-2 variants (15) it is important to assess whether their mutations are found in proximity to the found peptides, and whether these

mutations have a role in increasing viral transmissibility. We discovered five such variant mutation sites present in proximity to at least one of our peptides (Table 3) that are present in current prominent variants (Alpha (B.1.1.7 _UK), Beta (B.1.351_South Africa), Delta (B.1.617.2_India), and Mu (B.1.621_Colombia)).

The L452R and G446D mutations present in the delta variant (16–20) were found within the overlapping two selected epitopes S_0445 and S_0449. Although these mutations are not believed to directly affect the ACE2 binding activity of the virus, they are located in a peptide stretch that has been identified as an epitope for neutralizing antibodies and are thought to help inhibit neutralizing activity **(28)**. Another mutation, R346K, is found within the S_0343 peptide sequence. This mutation is linked to the Mu Variant **(19)** and is predicted to help with escape from certain classes of antibody. Findings of recent studies suggest that the L452R, G446D, and R346K mutations may have a potentially synergistic effect in escaping immune response, and may increasingly enhance infectivity when found together **[16, 28]**. Another set of mutations, P681R and P681H, are both located within the peptide of interest S_0671. The two mutations, which are commonly found in the Delta and Alpha mutants respectively, are mutations of the polybasic insertion sequence PRRAR, which has been identified as the S1/S2 furin cleavage site that appears to be completely unique to SARS-CoV-2 among closely related CoVs (17, 21-22). Cleavage of this site gives RBD flexibility to change between an open and closed conformation before ACE2 Binding, and is thought to be essential in conformational changes prior to viral host cell insertion (8, 14–16, 24). The P681R mutation found in the Delta variant is thought to enhance furin cleavage function **(30)**, most likely contributing to the increased infectivity of the Delta variant. Due to the significance of this mutation in the Delta variant, the S_0671 peptide may be an epitope of particular interest for future studies. Assessing whether antibody binding to the mutated linear epitope is observed could help assess whether the mutation may also have a more direct role in the enhanced viral escape of the Delta variant. The peptides found to be significant epitopes in our infected patient group, S_1141 and S_1247-S_1251, are located in the S2 subregion which is thought to be a region with less evolutionary pressure (26). The peptides do not appear to contain any common mutations observed in the currently relevant variants.(15).

It is of interesting note that none of the nine epitope sequences with significant IgG binding identified in the infected group were present in the N-terminal domain region of the spike protein. These findings are corroborated by previous epitope mapping studies utilizing a proteome wide microarray that have identified few to no potential epitopes in this region (5, 7, 11)**.** The NTD region is thought to be a particularly important target of neutralizing antibodies (27, 28) and contains numerous mutations thought to help increase the infectivity of current variants (18, 27). It is possible that there may be a higher proportion of conformational epitopes or epitopes that elicit antibody binding later on in the immune response.

3.3 Antibody Binding Profile Comparison of Vaccinated and Infected Individuals

Our analysis of antibody immunoreactivity to the SARS-CoV-2 protein revealed evident differences between the antibody binding activity of the infected group and vaccinated

individuals (serum collected 6-44 days after receiving second dose). It would appear that IgG antibody binding was significantly higher in infected individuals than those that were vaccinated. While the vaccinated group did display higher IgG binding than the negative control group, the binding was not high enough to meet our criteria for any individual peptide. Previous studies have shown that vaccinated individuals produce a more robust and longer lasting antibody response than those previously infected and unvaccinated (18, 29). It is of note that as many of the acutely infected samples are collected during the time period (2-3 weeks after infection) where antibody production during infection is thought to peak regardless of symptom severity (18). In addition, the symptom severity of some of the infected patients were unknown, so the portion of infected patients with more severe symptoms, which has shown to correspond to higher antibody production (42), cannot be accounted for. So some of the comparisons between the vaccinated and infected groups are limited, as the immune response produced by a mildly symptomatic individual and severely symptomatic individual can vary dramatically. However, it would appear that recently infected individuals appear to have more significant linear IgG epitopes than vaccinated individuals.

Although our results indicated that the vaccinated individuals did not have SARS-CoV-2 S protein epitopes that showed consistent binding, there were still some peptides that demonstrated the ability to potentially discriminate between vaccinated and naive individuals (S_1159 and S_1247-S_1251). These peptides did not meet the fold change criteria to be identified as significant in the study, so their potential sensitivity when incorporated into other assays is unknown. These peptides are well conserved amongst the human infecting CoVs which means the possibility this enhanced binding may be non-specific cannot be ruled out. Still, due to the potential uses of these peptides as markers to differentiate between vaccinated and naive individuals further assessment of these peptides could prove useful.

The lack of significant IgA response within the spike region was somewhat surprising, as previous studies had successfully identified epitopes within the spike protein utilizing peptide microarrays (11). Our ELISA data revealed relatively low IgA levels among some of the infected samples. The severity of symptoms of our tested samples ranged from asymptomatic to moderate according to the information provided by Raybiotech (Atlanta, GA). A potential source of these lower IgA levels may be tied to a portion of the serum samples coming from asymptomatic and mildly symptomatic patients. IgA epitopes identified in previous peptide microarray studies (7) found that IgA epitopes were mainly identified amongst their severe symptom patient group. Since the quantity of IgA in serum is only one tenth of IgG, it is possible that levels of IgA present in some screened infected samples with milder symptoms was not high enough to discriminate from the naive samples. It has been observed that the amount of IgA antibodies reactive to the SARS-CoV-2 spike protein can vary dramatically among infected individuals, and significant decreases in IgA levels can be observed as early as four weeks after infection (31). In summary the lack of IgA binding observed may be due to the relatively low levels of IgA found in serum compounded with the lower overall antibodies levels observed in mild and asymptomatic patients, which make up a portion of the infected samples used in the study.

Emerging evidence confirms that vaccine- or infection elicited antibodies raised against the Wuhan virion spike proteins are likely to have reduced activity against circulating variants, especially with the mRNA and adenoviral vaccines currently being used. Our microarray studies should indicate if such breakthrough infections by current delta and omicron variants in previously vaccinated persons will elicit similar IgG/IgA antibody patterns as the earlier infections. Such microarray analyses may reveal, however, that revaccinating these breakthrough infectious individuals, as well as other naturally infected persons should not only enhance maximal antibody titers against already prior existing spike epitopes, but more significantly, would likely broaden their antigenic exposures, not only to the sub-optimally (e.g., delta, omicron) recognized variants spike domain epitopes, but to other enhancing antiviral targets. Importantly this follow-on vaccination, could not only increase antibody titers, but would likely result in better affinity maturation and $CD4^+/CD8^+$ T-cell protection as well (47, 48).

Using a SARS-CoV-2 peptide microarray to perform IgA epitope profiling of sera may be difficult due to the limitations previously described without significant purification and concentration of IgA from samples. However, the potential for studying IgA epitopes of SARS-CoV-2 may still be viable with the use of saliva, a bodily fluid with a high content of IgA. Previous work using the peptide microarray to study the epitopes of other viral targets have shown the IgG antibody profiles of blood and saliva were similar (45). Salivary IgA has shown potential as a reliable biomarker for SARS-CoV-2 infection and a correlation between symptom severity and levels of salivary IgA have been previously demonstrated (46). Developing a method to treat microarrays with saliva may help to study IgA binding activity to SARS-CoV-2 epitopes. Previous attempts to directly treat microarrays with saliva are limited, but we have managed to obtain reproducible results screening processed saliva at low dilutions. Our ongoing study of IgA and IgG binding of saliva samples from previously infected individuals has demonstrated antibody binding to some epitopes identified in this report. These results are preliminary, with much further analysis and validation required to draw any conclusions. Yet the screening of saliva samples may be helpful with efforts to perform IgA epitope profiling of the SARS-CoV-2 proteome and lead to development of saliva-based diagnostics, immunity determinations, and therapeutics. This could also include detecting the immune response from the presence of the virus in the upper respiratory system within asymptomatic and pre-symptomatic individuals, possibly prior to detection by other methods.

Our comparison of the SARS-CoV-2 spike protein epitope profiles of vaccinated and infected individuals has led us to identify nine peptides with distinctly high IgG antibody reactivity in recently infected individuals. These nine peptides could potentially be implemented in diagnostic tools that could test for the presence of protective antibodies or active infection. Peptides with the potential to identify vaccine-only individuals have been identified, but further assessment of the use of said peptides to discriminate between vaccinated and naive individuals is necessary. The peptides may also serve to study the effect of mutations found in variants have on antibody binding, information that is crucial as new variants continue to develop in the pandemic landscape.

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This study is supported by contract from the U. S. Defense Threat Reduction Agency MCDC2007-003

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Figure 1a.

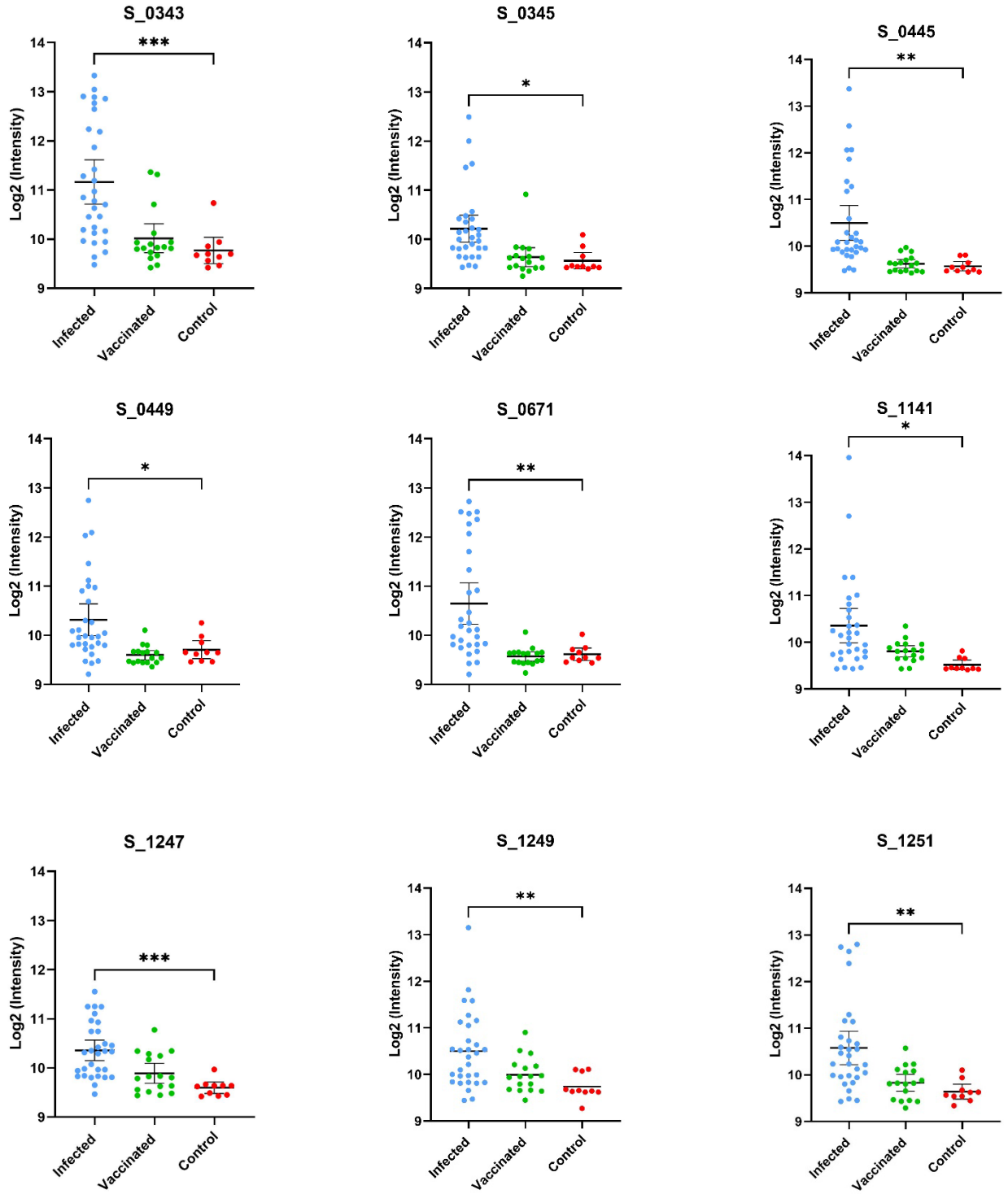

Figure 1b.

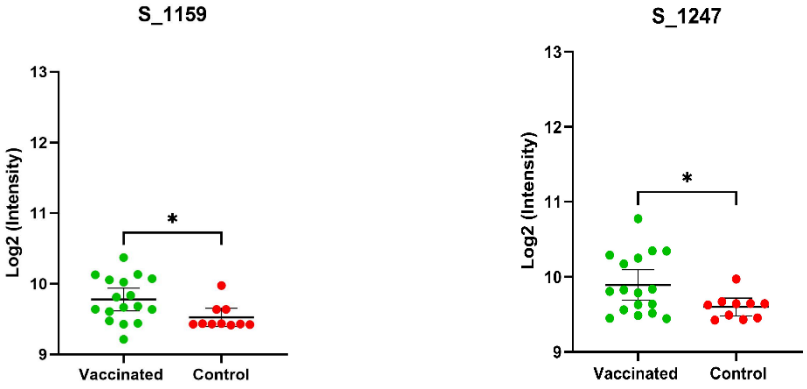

Figure 2

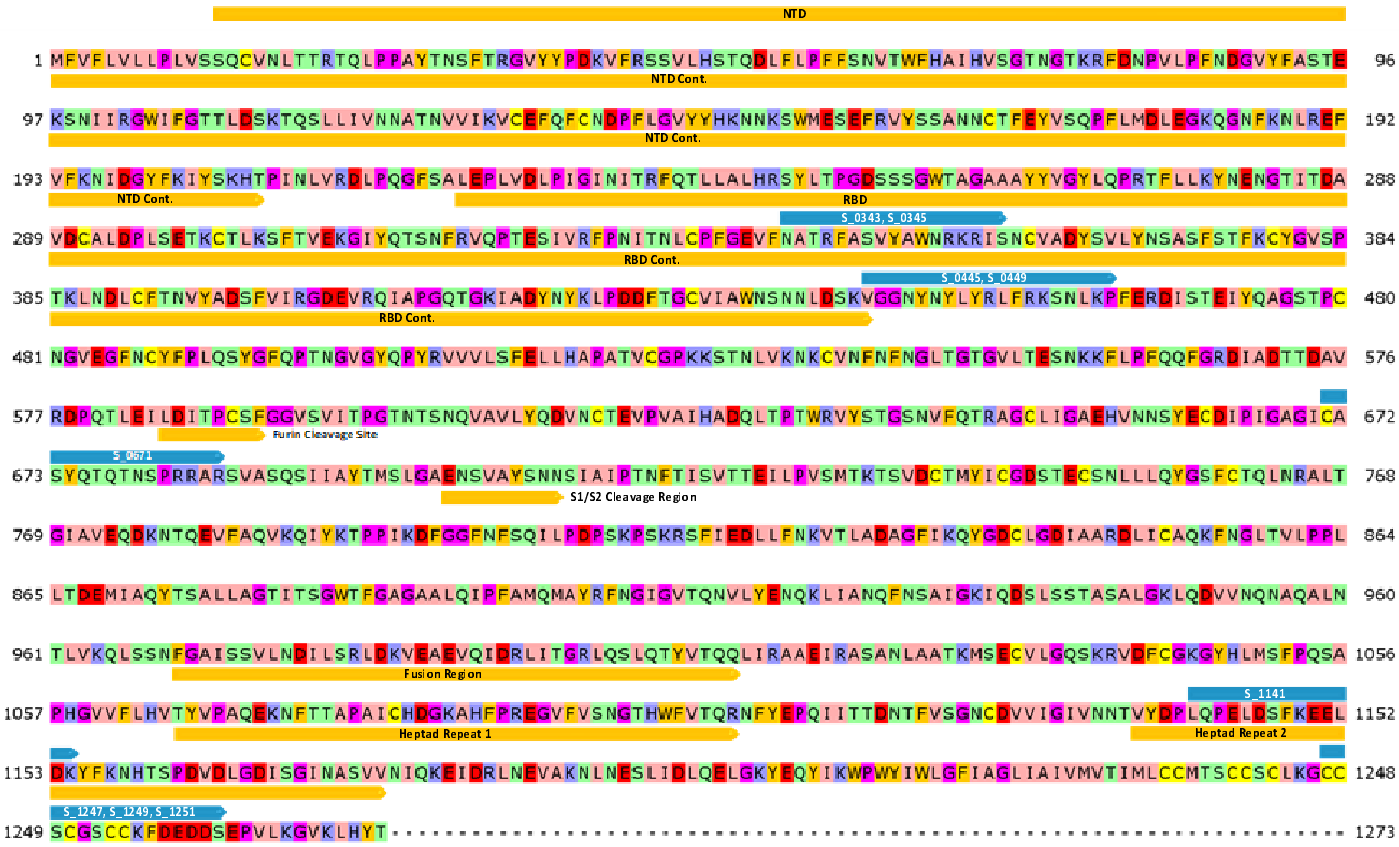

Figure 3

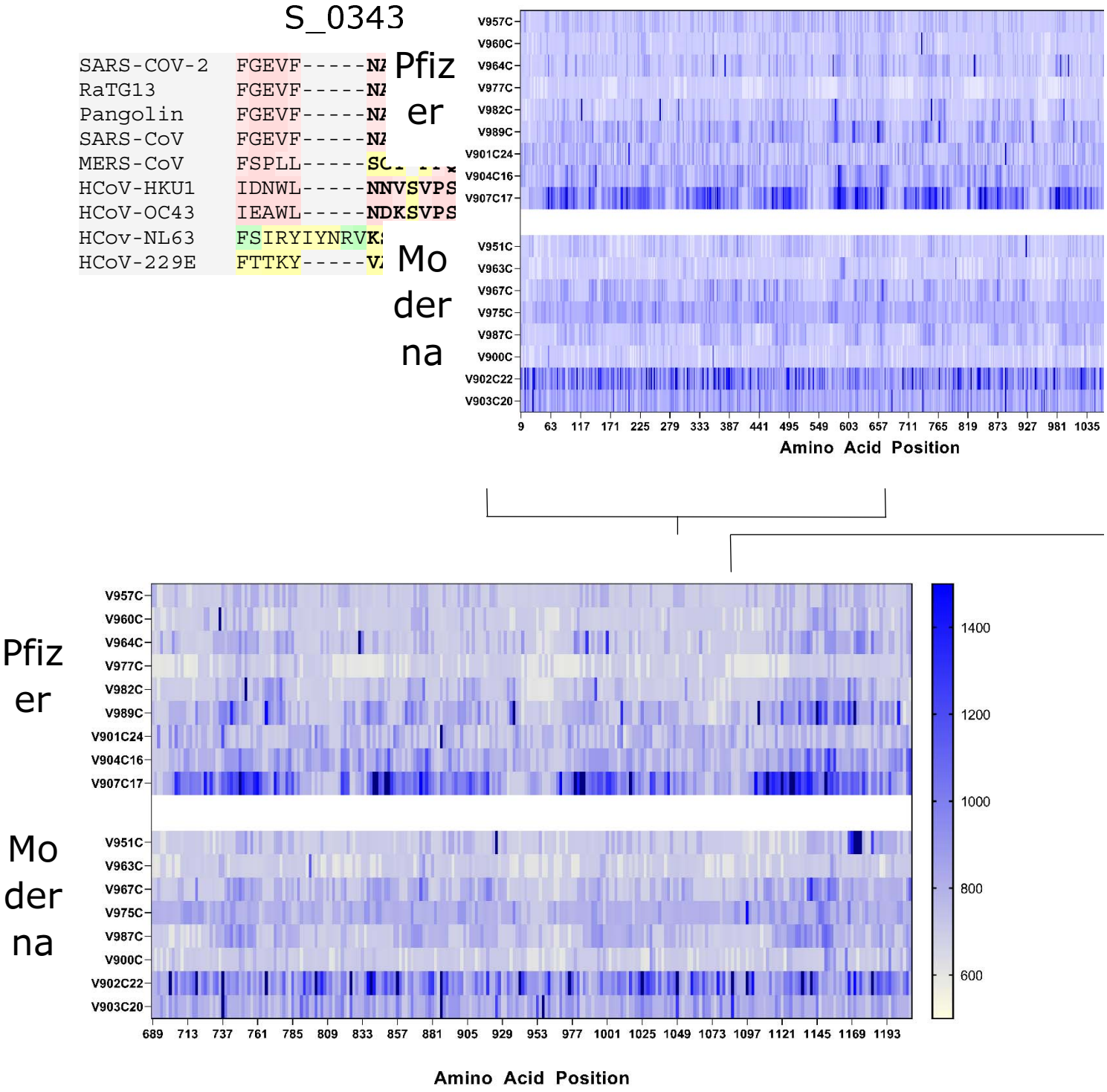

Figure 4.

BAD AVG GOOD

S_0343, S_0345

```
SARS-COV-2   FGEVF-----NATRFASVYA-WNRKRISNCV
RaTG13       FGEVF-----NATTFASVYA-WNRKRISNCV
Pangolin     FGEVF-----NATTFASVYA-WNRKRISNCV
SARS-CoV     FGEVF-----NATKFPSVYA-WERKKISNCV
MERS-CoV     FSPLL-----SGT-PPQVYN-FKRLVFTNCN
HCoV-HKU1    IDNWL-----NNVSVPSPLN-WERRIFSNCN
HCoV-OC43    IEAWL-----NDKSVPSPLN-WERKTFSNCN
HCov-NL63    FSIRYIYNRVKSG-SPGDSSWHIYLKSGTCP
HCoV-229E    FTTKY-----VAV-YANVGRWSASINTGNCP
```

S_0671

```
SARS-COV-2   I----GAGICASYQTQTNSPRRARSVASQ
RaTG13       I----GAGICASYQTQTNSRS----VASQ
Pangolin     I----GAGICASYQTQTNSRS----VSSQ
SARS-CoV     I----GAGICASYHTVSLLRS----TSQK
MERS-CoV     L----GQSLCALPDTPSTLTPRSVRSVPG
HCoV-HKU1    M----GSGFCIDYALPSSRRKRRGIS--S
HCoV-OC43    V----GSGYCVDYSKNRRSRGAIT----T
HCov-NL63    VMTYSNFGICADGSLIPVRPRNSSD----
HCoV-229E    VLTYSSFGVCADGSIIAVQPRNVSY----
```

S_0445

```
SARS-COV-2   NLDSKVGGNY-NYLYRLFRKSNL---KPF
RaTG13       HIDAKEGGNF-NYLYRLFRKANL---KPF
Pangolin     NLDSKVGGNY-NYLYRLFRKSNL---KPF
SARS-CoV     NIDATSTGNY-NYKYRYLRHGKL---RPF
MERS-CoV     HNLTTITKPL-KYSYINKC-SRL---LSD
HCoV-HKU1    LVNVTINNFNPSSWNRRYGFGSFNL---S
HCoV-OC43    AANVSVSRFNPSTWNKRFGFIEDSVFKPR
HCov-NL63    ----------------------------
HCoV-229E    ----------------------------
```

Figure 4 Cont.

S_0449

```
SARS-COV-2   KVGGNY-NYLYRLFRKSNL---KPFERDI
RaTG13       KEGGNF-NYLYRLFRKANL---KPFERDI
Pangolin     KVGGNY-NYLYRLFRKSNL---KPFERDI
SARS-CoV     TSTGNY-NYKYRYLRHGKL---KPFERDI
MERS-CoV     TITKPL-KYSYINKC-SRL---LSDRRTE
HCoV-HKU1    TINNFNPSSWNRRYGFGSFNL---SS---
HCoV-OC43    SVSRFNPSTWNKRFGFIEDSVFKPRPAGV
HCov-NL63    ----------------------------
HCoV-229E    ----------------------------
```

S_1141

```
SARS-COV-2   TVYDPLQPE--LDSFKEELDKYFKNHT
RaTG13       TVYDPLQPE--LDSFKEELDKYFKNHT
Pangolin     TVYDPLQPE--LDSFKEELDKYFKNHT
SARS-CoV     TVYDPLQPE--LDSFKEELDKYFKNHT
MERS-CoV     NLPPPLLGNSTGIDFQDELDEFFKNVS
HCoV-HKU1    IYLNNSIPN--LSDFEAELSLWFKNHT
HCoV-OC43    VMLNTSIPN--LPDFKEELDQWFKNQT
HCov-NL63    VELHTVIPD--YVDVNKTLQEFAQNLP
HCoV-229E    SELQTIVPE--YIDVNKTLQELSYKLP
```

S_1247, S_1249, S_1251

```
SARS-COV-2   SCLKGCCS-CGSCCKFDE--DDSEPVLK
RaTG13       SCLKGCCS-CGSCCKFDE--DDSEPVLK
Pangolin     SCLKGCCS-CGSCCKFDE--DDSEPVLK
SARS-CoV     SCLKGACS-CGSCCKFDE--DDSEPVLK
MERS-CoV     TNCMGKLK-CNRCCDRYE--EYDLEP-H
HCoV-HKU1    SACFS--K-CHNCCDEYG--GHNDFVIK
HCoV-OC43    TSCFK--K-CGGCCDDYT--GYQELVIK
HCov-NL63    GCCNCLTSSMRGCCDCGSTKLPYYEF-E
HCoV-229E    GFFSCFASSIRGCCESTK--LPYYDV-E
```

Table 1.

ID	Sequence	AA Position	Fold Change	p-value
S_0343	NATRFASVYAWNRKR	343-357	3.67	1.0E-3
S_0345	TRFASVYAWNRKRIS	345-359	1.51	4.5E-3
S_0671	CASYQTQTNSPRRAR	671-685	2.91	7.1E-3
S_0445	VGGNYNYLYRLFRKS	445-459	2.59	6.1E-3
S_0449	YNYLYRLFRKSNLKP	449-463	1.87	3.6E-2
S_1141	LQPELDSFKEELDKY	1141-1155	2.45	1.2E-2
S_1247	CCSCGSCCKFDEDDS	1247-1261	1.79	2.0E-4
S_1249	SCGSCCKFDEDDSEP	1249-1263	2.12	6.8E-3
S_1251	GSCCKFDEDDSEPVL	1251-1265	2.47	4.2E-3

Table 2.

Vaccine	Sequence	AA Position	Region
Moderna[1]	**DPLQPELDSFKEELD**	1135-1149	S2
Moderna[2]	**SCGSCCKFDEDDSEP**	1249-1263	S2, Intracell
Moderna	SFTRGVYYPDKVFRS	31-45	S1, NTD
Moderna Pfizer	**NATRFASVYAWNRKR**	343-357	S1, RBD
Moderna[1]	**LQPELDSFKEELDKY**	1137-1151	S2
Moderna[2]	**CCSCGSCCKFDEDDS**	1247-1261	S2, Intracell
Moderna	GTHWFVTQRNFYEPQ	1099-1113	S2
Moderna[1] Pfizer	**VYDPLQPELDSFKEE**	1133-1147	S2
Moderna	DVVIGIVNNTVYDPL	1127-1141	S2
Moderna	CLIGAEHVNNSYECD	651-665	S1
Moderna	NCDVVIGIVNNTVYD	1125-1139	S2

Table 3.

Mutation	Variant Found	Epitopes of Interest
L452R	*Delta*	*S_0445, S_0449*
G446D	*Delta*	*S_0445*
R346K	*Mu*	*S_0343, S_0345*
P681R	*Delta*	*S_0671*
P861H	*Alpha*	*S_0671*

Table S1.

Supplemental Figure S1.xlsx

Table S2.

Sample ID	Sex	Age	Days Post Vaccine	Vaccine Received
*V901A**	*M*	*32*	*N/A*	*N/A*
*V902A**	*M*	*34*	*N/A*	*N/A*
*V903A**	*M*	*47*	*N/A*	*N/A*
*V904A**	*M*	*37*	*N/A*	*N/A*
*V907A**	*F*	*48*	*N/A*	*N/A*
*V901C24**	*M*	*32*	*24*	*Pfizer*
*V902C22**	*M*	*34*	*22*	*Moderna*
*V903C20**	*M*	*47*	*16*	*Moderna*
*V904C16**	*M*	*37*	*16*	*Pfizer*
*V907C17**	*F*	*48*	*17*	*Pfizer*
V951	M	47	19	Moderna
V957	F	51	17	Pfizer
V960	F	25	28	Pfizer
V963	F	26	19	Moderna
V964	M	58	9	Pfizer
V967	M	66	17	Moderna
V975	M	67	44	Moderna
V977	F	36	10	Pfizer
V982	M	22	21	Pfizer
V987	M	62	20	Moderna
V989	M	24	6	Pfizer
V900	M	65	108	Moderna
SN205	F	22	N/A	N/A
SN206	M	18	N/A	N/A
SN207	M	47	N/A	N/A
SN210	M	28	N/A	N/A
SN212	F	39	N/A	N/A

Table S3.

Microsoft Excel Worksheet

Table S4.

Supplemental Table S5.xlsx

Descriptions:

Figure 1a.

Displayed are the Log2 normalized individual fluorescent intensity values of infected individuals within three weeks post symptom onset (n=18), individuals at five weeks post symptom onset (n=13), vaccinated (n=17), and control (n=10) sample groups. The peptide ID along with the results of the unpaired t-test comparing the infected and control groups can be found above the data. The results of the t-test comparing the fluorescent intensities of the infected individuals within three weeks post symptom onset and negative control groups values are displayed.

Figure 1b.

Shown are the two peptide sequences in which the mean $\log_2$ normalized fluorescent intensities of the vaccinated and control groups were found to be significantly different (p-value < .05) based on the results of an unpaired t-test. The ID and results of the t-test are displayed above. The vaccinated group was not found to have a fold change < 1.5, and thus were recognized as epitopes of highest interest based on our criteria.

Figure 2.

Depicted is the amino acid sequence of the SARS-CoV-2 spike protein. Blue arrows depict the locations of epitopes with significant IgG binding. Protein sequence is colored according to the Zappo amino acid color scheme (with brightness of some colors increased for clarity).

Figure 3.

Heat map of the whole SARS-CoV-2 spike protein (above) and S2 region (Below) displaying the raw fluorescent intensity values of vaccinated samples. The fluorescent intensity values are read at the 785nm wavelength, which corresponds to IgG binding activity. Sample IDs are displayed to the left of their respective columns. Individuals who received the Pfizer mRNA vaccine are displayed in the upper panels while those who received the Moderna mRNA vaccine are found in the lower panels. Amino acid sequence of entire spike protein is displayed in linear manner with amino acid positions denoted below.

Figure 4.

Shown are the aa alignments of the seven selected peptides of interest. Each alignment compares the sequence found in SARS_CoV-2 to the other 6 infected CoVs in addition to the closely related Pangolin and bat infecting Coronaviruses. Peptides IDs are displayed above their corresponding alignments and the color scale for the alignment can be found above.

Table 1.

Three peptides that met our criteria to be classified as potential epitopes amongst infected individuals. In first column are IDs, which were assigned based on the position of the first amino acid in the sequence. Amino acid sequences and their location in the spike protein sequence are

displayed in the second and third columns respectively. Fold change and p-values acquired was described in statistical analysis are found in the fourth and fifth columns.

Table 2.

Displayed are peptides whose mean fluorescent intensities were at least three standard deviations higher than the overall average intensities in either the Pfizer or Moderna vaccinated groups. Vaccine group in which the peptide met these criteria are displayed in the first column (no peptides were found to be unique to the individuals who received the Pfizer vaccine). The amino acid sequence of these sequences is displayed in the second column and those in bold are amongst or share significant amino acid identity with 1 of the 7 potential epitopes identified in the infected sample group. Amino acid position and domain/subregion of each sequence is displayed in the third and fourth columns.

[1] Part of the contiguous amino acid sequence VYDPLQPELDSFKEELDKY

[2] Part of the contiguous amino acid sequence CCSCGSCCKFDEDDSEP

Table 3

Listed are the five mutations found in proximity to at least one of our identified peptides of interest. From left to right the position of the mutation, the variants the mutation is found in, and the peptides of interest which contain that mutation are shown.

Table S1.

Patient information for 20 serum samples belonging to the infected sample group. Sample IDs were assigned by the company that collected the sample (either Raybiotech or Reprocell). Sex and age of patient are denoted in the second and third columns respectively. Date of positive test (either antigen or PCR) and date of sample collection as reported by either Raybiotech or Reprocell are list in the third and fourth columns. The sixth column contains patient symptom information, which was reported by those who collected the sample as either none, mild, moderate, or severe. Patients with no reported symptom data are stated as Unavailable. Samples that are shaded gray are the paired (pre-vaccine and post vaccine) samples.

Table S2.

Patient information of 17 vaccinated and 10 negative control serum samples. The first three columns contain sample IDs along with sex and age of the individual. Days after second vaccination serum sample was collected from patient is displayed in fourth column.

Manufacturer of vaccine received is located in the final column. Negative control samples were collected before discovery of SARS-CoV-2 and all were purchased from Raybiotech.

* Italicized samples come in pairs from same patient (e.g. V901A comes from same patient as V901C24 and so on)

Table S3.

Available patient symptom information as reported by Raybiotech.

Table S4.

Corresponding ELISA data for all samples. IgG and IgA readings were detected as described in methods section 1.4