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## Establishment and Verification of a SARS CoV2 Antibody Detection Assay Comparing Venous and Mitra Samples

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Establishment and Verification of a SARS CoV2 Antibody Detection Assay Comparing  
Venous and Mitra Samples

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Medicine  
at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. Jerold Woodward, Professor of Immunology

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2022

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## ABSTRACT OF THESIS

### Establishment and Verification of a SARS CoV2 Antibody Detection Assay Comparing Venous and Mitra Samples

During the COVID-19 pandemic in 2020, the need for highly-specific, wide-spread, and rapid serological testing surged. In this study, we showed very strong positive correlation between venous blood collection and Mitra home sampling kits for the detection of anti-spike IgGs using an in-house ELISA protocol (based on a protocol from Mt. Sinai). This study demonstrates the utility of using at-home, patient-centric testing to enhance the sero-surveillance methods currently in place for viral tracking and monitoring.

**KEYWORDS:** COVID-19, Serological Testing, ELISA, SARS CoV2, Mitra

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## CHAPTER 1. INTRODUCTION

During 2020, the rise of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2), a virus that causes COVID-19, brought great harm and destruction to the human population, killing 6.5 million people and rising worldwide<sup>1</sup>. According to the American Pandemic Preparedness Team, it is estimated that we will likely see an even stronger pandemic within the next decade and more to come after<sup>2</sup>. To ensure early detection and prevention of future pandemics, real-time population monitoring is required, including tracking viral variants, viral-infection monitoring, and epidemic analysis and forecasting. To do this successfully, highly sensitive serological testing must be readily available and performed at a rapid rate. In regards to controlling active pandemics, widespread epidemiological testing is needed in order to define disease control methods in real-time and to make data-driven policy changes<sup>3</sup>.

Coronaviruses (CoV), varying in size from 65 to 125 nm, are the largest RNA viruses currently known<sup>4</sup>. They are members of the genus *Coronaviridae*, pleomorphic RNA based viruses that are composed of crown-shaped peplomers<sup>5</sup>. SARS CoV2 shares considerable sequence homology with SARS-Coronavirus and the Middle East MERS-Coronavirus and is a rapidly spreading respiratory virus worldwide, originally defined in Wuhan, China<sup>5</sup>. Coronaviruses are classified based on the crown-like spikes present on their outer membrane into one of four main categories, alpha ( $\alpha$ -CoV), beta ( $\beta$ -CoV), gamma ( $\gamma$ -CoV), or delta ( $\delta$ -CoV)<sup>5</sup>. SARS-CoV-2 is classified into the  $\beta$ -CoV category due to it being composed of positive-strand RNA with a lipid envelop and being able to infect mammals<sup>6</sup>. Viral spike proteins are made up of two smaller subunits, S1 and S2. S1 protein is responsible for receptor binding as it contains the receptor binding domain

(RBD) and S2 contains the fusion machinery responsible for integrating the viral RNA into the host cell membrane<sup>8</sup>.

SARS CoV2 human-human transmission occurs through aerial droplets during conversation, breathing, sneezing, or coughing when two people are nearby<sup>5</sup>. During an active infection, SARS CoV2 circulates around the body to find healthy host cells. The spike protein on the outer membrane of the viral surface binds to its cognate receptor on the host cell, angiotensin-converting enzyme 2 (ACE2)<sup>5,7</sup>. ACE2 contains 805 amino acids. It is ubiquitous within the human body, but is overexpressed on cellular outer membranes of the intestine, heart, lungs, kidney, testis, and brain<sup>8</sup>. Due to SARS CoV2 spreading as aerial droplets, virus enters the human body through the upper respiratory tract's epithelial tissues and proceeds to the lower respiratory tract to infect alveolar and bronchial epithelium, cells that also overexpress ACE2<sup>8</sup>.

After ACE2 binding, the host cell's transmembrane protease serine 2 (TMPRSS2) cleaves the S1 and S2 subunits of the viral spike protein to allow fusion of the virus envelope to the host cell's plasma membrane and release of viral RNA into the cytosol<sup>7</sup>. The positive-strand RNA uses the host cell's protein synthesis machinery to create viral RNA polymerase and non-structural proteins needed for RNA replication<sup>7</sup>. The subsequent products of RNA replication and transcription are the protein subunits needed to construct more SARS CoV2 structures, including spike, envelope, membrane, and nucleocapsid proteins. The structural and accessory proteins are then transported to the Endoplasmic-Reticulum Golgi Intermediate Compartment (ERGIC), where they are packaged into virions to be secreted back into the body's circulation to infect other host cells<sup>7</sup>.

As the infection progresses and epithelial cells are damaged and lysed by the virus, the immune system begins to rapidly respond<sup>9</sup>. Due to this rapid response, innate immune cells overproduce pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , TNF), small proteins that can have paracrine, autocrine, or endocrine functions on other immune cells<sup>10</sup>. When maintained within proper range, these cytokines are crucial for proper immune system function and coordination. The acute rise of pro-inflammatory cytokines during an active SARS CoV2 infection results in the rapid influx of immune cells to the site of infection, leading to damaged tissues and respiratory distress<sup>10</sup>. Early detection and proper treatment of this so called “cytokine storm” can lead to better clinical outcomes for SARS CoV2 patients.

In order to effectively defeat the SARS CoV2 virus and limit human-human transmission, mechanisms of host cell entry or viral replication must be prevented. Coronaviruses and their possible vaccines have been studied for many years prior to the SARS CoV2 pandemic in 2020<sup>11</sup>. In 1984, a group at Harvard University in the United States synthesized RNA polymerase enzymes to make synthetic messenger RNA (mRNA) in the laboratory<sup>5</sup>. This was a major break-through in studying gene activity and function. In the 1990s, synthetic mRNA was used as a treatment in rats for cancer and influenza, but it was not until 2005 when mRNA was able to be genetically modified to be more stable in humans, leading to increased federal funding and widespread mRNA vaccine research<sup>11</sup>.

The first two mRNA-based vaccines FDA-approved for large-scale use by humans were for SARS CoV2 in 2020, produced by Pfizer-BioNTech and Moderna<sup>11</sup>. There are many public skeptics and a number of conspiracy theories concerning RNA-

based vaccines that arose in response to the rapid timeline of vaccine development, however, scientists and researchers were able to use the years of prior MERS-CoV and SARS-CoV vaccine research to adapt protocols to the SARS CoV2 virus and produce an effective vaccine to curb transmission rates<sup>11,12</sup>.

To develop effective mRNA-based vaccines, researchers exploited the virus' need to bind to ACE2, with the goal of preventing viral entry into the host cell<sup>13</sup>. To do this, the mRNA sequence of the SARS CoV2 spike protein was isolated in a laboratory and packaged into a lipid nanoparticle<sup>13</sup>. Putting the recombinant mRNA spike protein sequence inside of a lipid nanoparticle helps better stimulate the innate immune system's professional antigen presenting cells (APCs) and prevents proteolysis of mRNA *in vivo*<sup>14</sup>. After fusion of the lipid nanoparticle to the host cell membrane, the mRNA sequence is taken up and transported to the ribosome for translation<sup>13</sup>. The subsequently produced spike protein molecules will then be presented on the host cell's surface by major histocompatibility complexes (MHCs) for recognition by T cells. MHCs are present on almost every cell inside the human body and are critically important for presenting intracellular and extracellular peptides for immune-surveillance, determining if cells are healthy or need to be destroyed<sup>15</sup>. MHCs are highly variant and extremely difficult for pathogens to evade due to their polygenic (high number of genes) and polymorphic (multiple variants within a population) features<sup>16</sup>.

After vaccination or viral infection, viral proteins are presented by APCs in peripheral lymphoid tissue. Following innate immune cell stimulation and expression of co-stimulatory molecules, CD4+ or CD8+ T-cells differentiate to carry out their effector functions specific to spike protein or other viral proteins<sup>16</sup>. In peripheral lymphoid tissues

(lymph nodes and spleen), B-cells also encounter spike protein antigens to become activated with the help of follicular dendritic cells and T<sub>FH</sub> cells. B-cells can then differentiate into either plasma B-cells to rapidly produce high-affinity antibodies (Immunoglobulins, Ig) or memory B cells, enabling a more rapid response for future infections<sup>16,17</sup>. Antibodies can be separated into five classes based on their constant regions and effector functions--IgM, IgD, IgG, IgA, and IgE. IgG antibodies have several subclasses and is the most abundant isotype circulating in serum<sup>18</sup>. The newly synthesized IgG spike protein-specific antibodies from the plasma B-cells help neutralize SARS CoV2 by binding to spike protein molecules and blocking the interaction with ACE2 on the host cell<sup>13</sup>. In addition, antibodies bound to pathogens enhance the binding of phagocytic immune cells to neutralized virions to help clear and dispose of the virus. Phagocytic immune cells (neutrophils and monocytes) contain Fc receptors that enable binding to IgG antibody constant regions, thus promoting uptake of Ab-coated virus<sup>17</sup>.

The presence of spike protein antibodies in serum are one of the biological markers that can help determine if a patient has been recently infected with SARS CoV2. To detect and quantify the presence of spike protein antibodies in serum, serological tests such as an enzyme-linked immunosorbent assay (ELISA) can be conducted.

The development of the ELISA was a major breakthrough for the field of Immunology, simultaneously invented by Dr. Perlman and Dr. Schuurs through modifications of the existing radioimmunoassay (RIA)<sup>19</sup>. ELISAs are highly sensitive serological tests that can be used to quantify small substances such as proteins, antigens, antibodies, hormones, and glycoproteins using very small sample volumes<sup>19</sup>. Due to their high sensitivity, signal amplification, low cost, and potential for automation, ELISAs

have broad use in immunology research and diagnostics<sup>20</sup>. ELISA plates are made of polystyrene and contain a certain number of wells that bind protein molecules with high-affinity. In regards to screening antibody levels in serum, an indirect ELISA can be used. Using this example, each well is coated with recombinant target protein and then blocked with an agent (such as ovalbumin, BSA, or aprotinin) to prevent the binding and interaction of any unwanted antibodies<sup>19</sup>. Unknown serum samples then can be placed into the wells and if the serum contains antibodies specific to the coated protein of interest, they will bind with high-affinity. Following a wash-step, an enzyme-conjugated secondary antibody is placed into the wells to allow interaction with any bound antibody<sup>19</sup>. The addition of a substrate that interacts with the conjugated enzyme causes a solution color change in the wells based on the amount of enzyme present, directly correlating to the amount of bound primary antibody<sup>19</sup>. Using a spectrophotometer, ELISA plates can be read and quantified to give highly-accurate antibody titer levels for unknown serum samples.

During the early phases of 2020, delayed diagnostic pipelines and asymptomatic SARS CoV2 infections were negatively affecting the ability to determine viral prevalence rates in Kentucky and around the World<sup>21</sup>. The need for highly-specific, wide-spread, and rapid serological testing surged. Conventional serological sample collection methods that require venous phlebotomy from patients, especially in an uncontrolled pandemic, do not effectively meet the demand for urgency. Rapid testing and properly defined viral prevalence rates allow experts to monitor trends of infection in the general population, stop geographical spread, and determine potential associated risk factors of the disease<sup>22</sup>.

Major rate limiting steps must be addressed with conventional serological sample collection protocols to better respond to future pandemics and diseases. Currently, limitations of high-scale serological testing include the need for a large number of healthcare professionals trained in venipuncture, patients having to schedule appointments and come into clinics, proper sample shipping, and the cost of labor needed in a research laboratory to properly process and store samples for testing<sup>23</sup>. In rural/remote areas affected by healthcare disparities, limitations with collection and testing can widen these disparities even further during an active pandemic.

In the early stages of a pandemic, it is important for people to stay at home and limit contact with others as much as possible to decrease the spread of disease. The development of highly sensitive mail-in sampling kits and serological assays would relieve the pressure on healthcare facilities and increase access to testing, not only for SARS CoV2, but any future virus. Using a Mitra micro sampler, with Neotryx's volumetric absorptive microsampling (VAMS) technology, we tested if anti-spike IgG antibody titers of capillary blood samples were comparable to IgG antibody titers from venous blood samples. Comparable results would remove the need for venous blood sample collection, have major implications for how we manage the rise of future pandemics, and will help enhance population disease serosurveillance protocols currently in place.



## CHAPTER 2. MATERIALS AND METHODS

### 2.1 Safety/Protocol Statement

This study was performed under an approved IRB protocol: #60876, Covid-19 Antibodies in Finger Stick Capillary Samples. Biosafety approval was obtained from the University of Kentucky's Institutional Biosafety Committee to work with blood samples from positive COVID-19 patients. In order to protect laboratory staff, proper PPE (lab coats, protective eyewear, disposable gloves) were worn, blood was centrifuged using rotor buckets with caps, and all samples were manipulated in a certified Biosafety Cabinet prior to heat inactivation.

### 2.2 Participants and Sample Collection

20 volunteers from Kentucky, varying in age, were recruited for this study and divided into two groups. Group 1 was composed of 10 healthy participants that previously received a positive test for SARS CoV2 (i.e. recovered) and group 2 was composed of 10 healthy participants with no known previous exposure or symptoms. The prior SARS CoV2 infection status was self-reported. None of the subjects were vaccinated. Subjects were de-identified and assigned a sample number depending on their group (FS-01 - FS-10 in group 1 and FS-11 - FS-20 in group 2).

Subjects were scheduled for an outpatient venous and capillary phlebotomy appointment with a clinician at the University of Kentucky's Center for Clinical and

Translational Science. Prior to the appointment, subjects were tested for an active SARS CoV 2 infection via PCR and it was required that the negative control group produce a test negative.

Venous blood samples were collected into a 4 mL BD vacutainer CPT tube. Capillary blood samples were collected using VAMS (Mitra Collection Kit; Neoteryx, CA, USA). To obtain capillary blood, a contact-activated lancet was used to prick the subject's fingertip. Approx. 20 uL of capillary blood was absorbed onto each Mitra tip, collecting two in total.

### 2.3 Sample Storage

Upon collection, venous and capillary samples were sent directly to the Human Immune Monitoring Lab (Biosafety-Level 2+) where they were immediately processed at room temperature (20-25°C). Venous blood sample CPT vacutainers were centrifuged for 30 minutes at 2850 rpm (brakes disengaged) to separate the serum and red blood cells. After centrifugation, serum plasma was extracted from the top layer of the vacutainer and dispensed into 5 ml FACS culture vials and heat-inactivated at 56°C for 30 min. Serum was then transferred into freezer vials and stored at -80°C until needed for ELISA assay. Capillary blood samples remained dehydrated on Mitra tips in sealed packages at ambient temperature until needed for testing.

## 2.4 Mitra Tip Hydration

One day prior to the ELISA, capillary Mitra tips were placed into 1 ml microcentrifuge tubes containing 200 uL of 1X PBS (ensuring each tip was fully submerged) and left to hydrate overnight at 4°C. On the day of the ELISA, capillary VAMS samples were removed from storage and directly used in subsequent ELISA assays (assuming a starting dilution of 1:10, 20 uL of dehydrated blood into 200 uL of 1X PBS). Any hydrated samples not used in testing were placed at -80°C until needed further.

## 2.5 ELISA Plate Preparation

One day prior to running the ELISA, all plates were coated with recombinant SARS CoV2 full-length spike protein in preparation for the assay. Spike protein was diluted to 2 ug/ml from 2 mg/ml stock solution with 1X PBS. 50 ul of 2 ug/ml spike was added to each well of a clear flat-bottom immuno nonsterile 96-well plate [Immulon 4HBX Thermo Scientific™ Cat # 3855 or equivalent] (ensuring the bottoms were completely covered in solution). Plates were then covered with plastic and stored at 4°C overnight to allow for protein binding.

## 2.6 ELISA Plate Blocking

On the day of the ELISA, the plates were removed from storage and were washed with 1X PBS 0.1% tween 20 three times at 200 ul/well using an Aquamax 2000 plate washer.

After washing, any remaining wash solution was discarded and 200 ul of 3% (w/v) non-fat dry milk in 1X PBS 0.1% tween buffer (Omniblok™ non-fat dry milk from Americanbio Inc; Cat # AB1010901000) was added to each well for blocking. Plates were then incubated at ambient temperature for 1 hour.

## 2.7 Sample Preparation/Loading

Capillary VAMS tips were removed from 4°C storage and serum samples from -80°C to let thaw. Once serum was thawed, a 1:10 dilution in 1X PBS was made for each sample. To prepare the standard curve, a 25 ug/ml dilution of Genscript anti-spike RBD (HC2001, monoclonal antibody) in 1X PBS was made.

After discarding the blocking buffer, 120 ul of 1% (w/v) non-fat dry milk in 1X PBS 0.1% tween buffer was added to all wells with an additional 42 ul in the top row of each plate. Samples were then loaded in duplicates in the top row of the plates adding 18 ul /well of STD curve anti-spike RBD (25 ug/ml), 1:10 serum samples, and 1:10 Mitra samples. Using a multichannel pipette, the top rows were mixed thoroughly and 60 ul was then transferred down to the next row, repeating this process for 7 rows and discarding the final 60 ul from row 7. Row 8 was left blank. Plates were incubated at ambient temperature for 1 hour to allow for primary antibody-protein binding.

## 2.8 Detection Antibody Loading

Plates were washed with 1X PBS 0.1% tween 20 three times at 200 ul/well using an Aquamax 2000 plate washer. A 1:1500 dilution of Goat anti-mouse IgG-HRP (50% glycerol stock Jackson ImmunoResearch, 115035003) was made with 1% (w/v) non-fat dry milk in 1X PBS 0.1% tween buffer. 50 ul/well was then added to each plate and incubated at ambient temperature for 1 hour to allow for secondary antibody-primary IgG antibody binding.

## 2.9 ELISA Plate Development and Reading

Plates were washed with 1X PBS 0.1% tween 20 three times at 200 ul/well using an Aquamax 2000 plate washer. HRP-substrate (SIGMAFAST™ OPD Sigma-Aldrich: Cat#P9187 or equivalent) was made by dissolving one urea hydrogen peroxide tablet into 20 ml of dH<sub>2</sub>O. Once dissolved, an OPD (*o*-phenylenediamine dihydrochloride) tablet was added to the solution and vortexed. After discarding any remaining wash solution in the plates, 100 ul of the OPD solution was added to all wells of each plate and developed at ambient temperature for 10 min. After 10 min, 50 ul/well of 3.0 M Hydrochloric Acid (Fisher Scientific: Cat#S25856, or equivalent) was added to stop the enzyme-substrate reaction. Plates were then immediately scanned and read at a 490 nm wavelength on a spectrophotometer.

## 2.10 Data Analysis

For each plate, the average of the negative controls (blank OD values) plus three times the standard deviation of the mean determined the level of background for each plate. Any sample with an optical density value 2X above the calculated background was considered positive for anti-spike IgG. Venous and capillary VAMS antibody titer curves were plotted in duplicates for comparison. Correlation analysis was conducted by averaging and plotting the optical density duplicates of each positive sample at a 1:100 serum dilution factor. As a positive control to ensure each ELISA was performed under optimal conditions, a standard curve using anti-spike RBD monoclonal antibody at a known concentration (25 ug/ml) was established for each plate.

## CHAPTER 3. RESULTS

### 3.1 Agreement of Capillary VAMS and Venous Anti-Spike IgG Antibody Titers

It has been demonstrated previously that capillary blood sampling shows major promise for the future of serological testing<sup>21,23,24</sup>. In support of these findings, our study demonstrated the utility of capillary blood sampling with the development of a highly qualitative and quantitative SARS CoV2 ELISA protocol (based on the Mt. Sinai SARS CoV2 ELISA protocol<sup>25</sup>).

To determine the correlation of the reciprocal serum dilution curves between capillary VAMS and venous sampling, all OD values were graphed in duplicates (Figures 1A, 1B, and 1C). For better graphical visualization of sample correlation, subjects were separated based on whether their max OD reading was high ( $>1.5$ ), medium ( $>0.5$ ), or low ( $<0.5$ ). There was excellent agreement of the dilution curves between venous and Mitra sampling for all levels of positivity. Importantly, we performed the comparison of venous and Mitra sampling for a particular individual within the same ELISA assay. A standard curve for every plate of each ELISA was produced by plotting the OD values of the positive control (anti-RBD) (Figure 2). For those assays using multiple plates, there was excellent agreement of the standard curve between plates, indicating that all intra-assay comparisons were valid.

To determine the degree of correlation between venous and Mitra sampling, we compared anti-spike IgG optical density absorbance values in capillary VAMS and venous samples. We averaged the duplicates of each positive sample at a 1:100 dilution and plotted the data on a scatter plot. Using 13 positive test subjects, we found strong

correlation between the two sampling methods ( $R= 0.985$ ) (Figure 4). These results provide a strong statistical demonstration of the correlation between venous and Mitra sampling for the measurement of anti-spike IgG antibody.

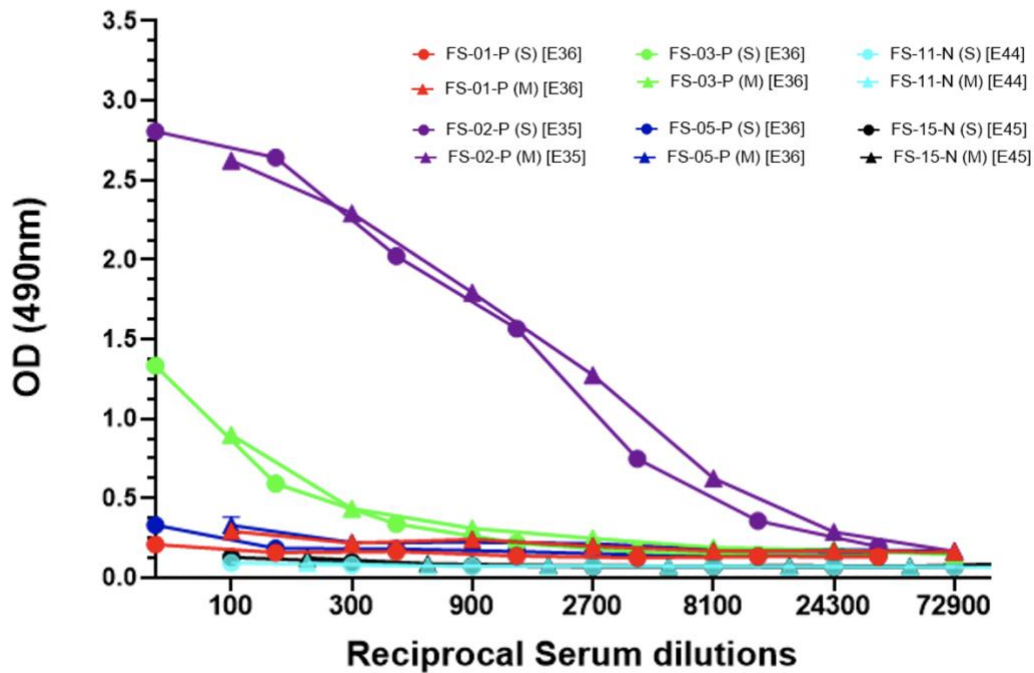
### 3.2 Antibody Titers of Positive and Negative Subjects.

Antibody titers were calculated based on the serum dilution curves shown in Figures 1A, 1B, and 1C. We separated the titers of the 20 subjects into two groups. Group 1 was composed of 10 healthy participants that previously received a positive test for SARS CoV2 (i.e., recovered) (self-reported) and group 2 was composed of 10 healthy participants with no known previous exposure or symptoms. Three out of 10 subjects in group 1 showed a strong titer consistent with prior SARS CoV2 infection while 7/10 showed a weak titer from both venous and Mitra samples (Figure 3A). Overall, the titers of group 1 were significantly higher than those of group two (Figure 3B), consistent with their SARS CoV2 negative status. Out of the 9 tested subjects in group 1, all 9 had OD values above the assigned background for that assay, meaning they were positive for serum anti-spike IgG antibodies. Out of 9 subjects in the negative control group, 3 had OD values above background while 6 did not meet the assay positivity threshold. We cannot exclude that these three subjects had a prior asymptomatic SARS CoV2 infection.



### 3.3 VAMS Antibody Stability at Ambient Temperature

Prior studies have shown antibody stability on DBS filter paper for more than 6 months, but due to VAMS being a newer sampling system, antibody stability is still being tested<sup>26</sup>. Upon receiving the dehydrated capillary VAMS samples from the University of Kentucky's Center for Clinical and Translational Science, we stored them at ambient temperature sealed in zip-lock bags. Due to receiving the samples ahead of when the SARS CoV2 assay was optimized, they were stored for varying number of days before being hydrated for use (Table I). While our original intent of the project did not include testing antibody stability, the results of our study show that antibodies were stable on Neoteryx's capillary VAMS kits dehydrated at ambient temperature for up to 126 days. Capillary VAMS antibody stability is proven through the IgG titer positive correlation with venous sampling shown in Figure 4.



**Figure 1A Reciprocal Serum Dilutions 1 of 3.** Paired serum (S) (circles) and capillary VAMS (M) (triangles) from 20 subjects were compared in a SARS CoV-2 ELISA measuring serum spike IgG antibody concentrations. OD values (read at 490 nm) were plotted with their corresponding reciprocal serum dilution to create a spike IgG dilution curve. The number listed next to each sample represents the ELISA assay they were tested in and their corresponding standard curves can be found in Figure 2. The suffix after the subject number denotes whether they were previously positive (P) or negative (N) for SARS CoV2.

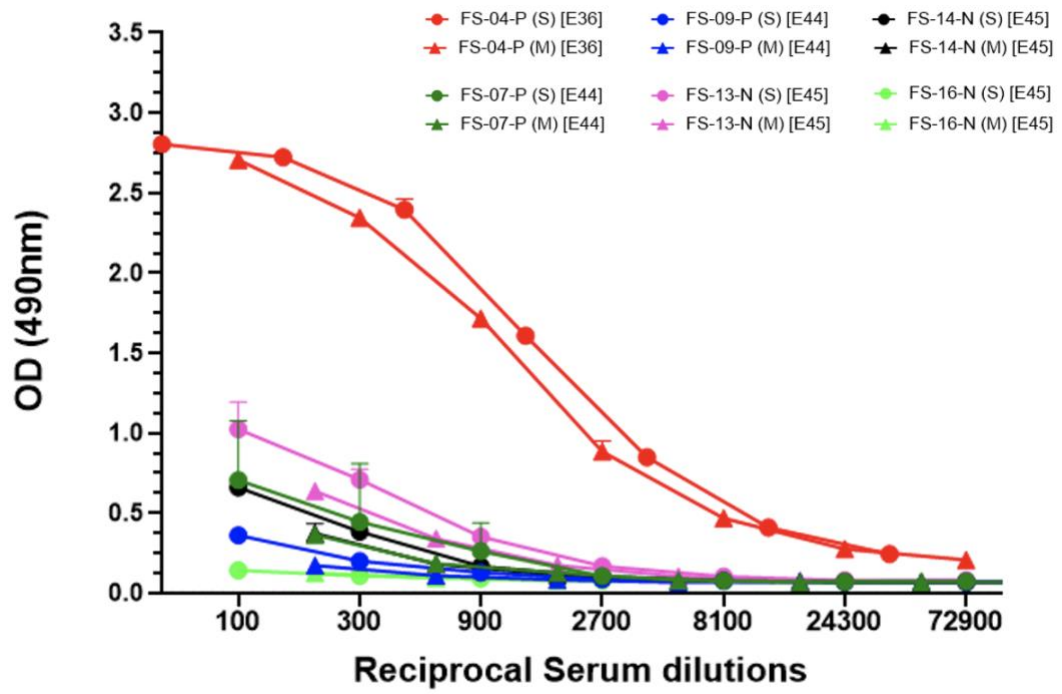


Figure 1B: Reciprocal Serum Dilutions 2 of 3.

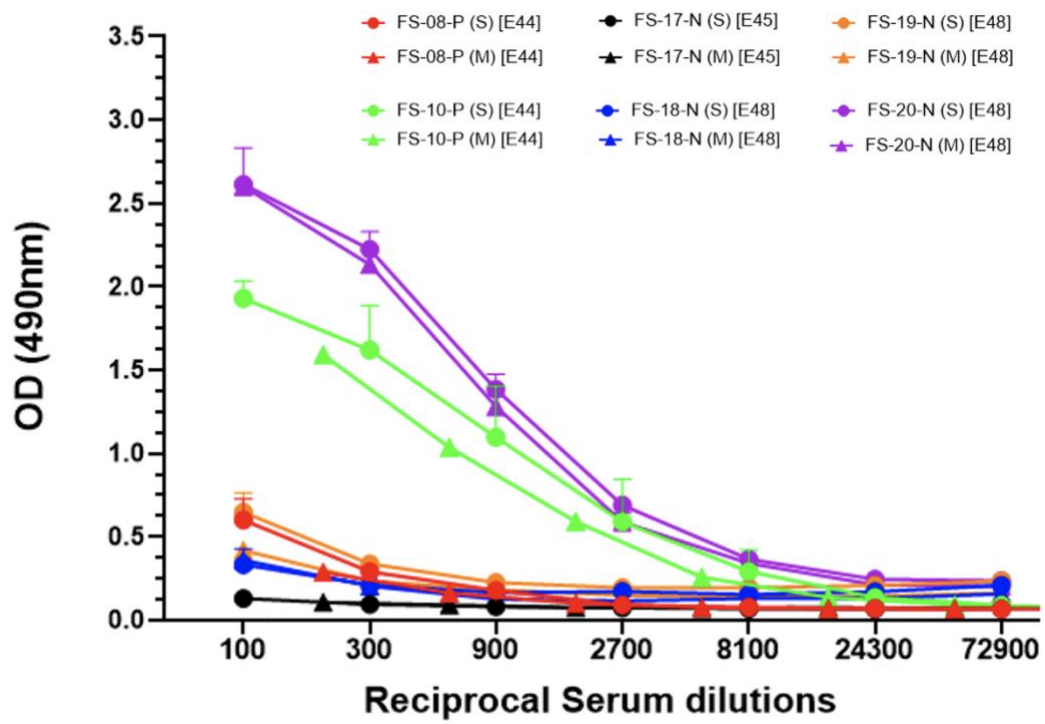
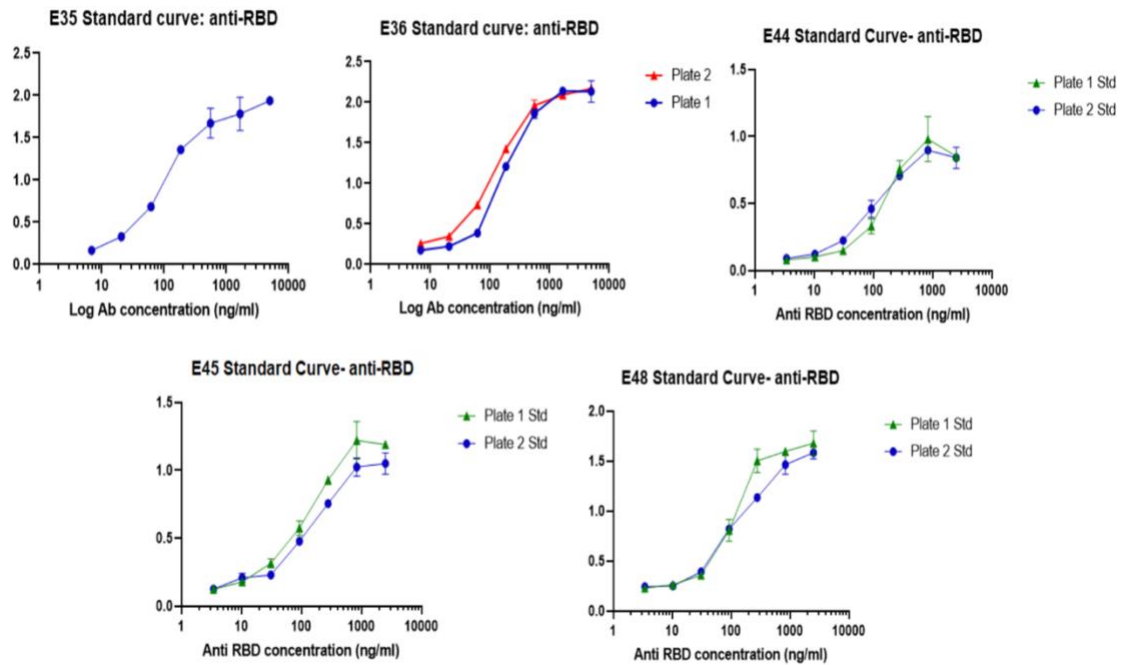
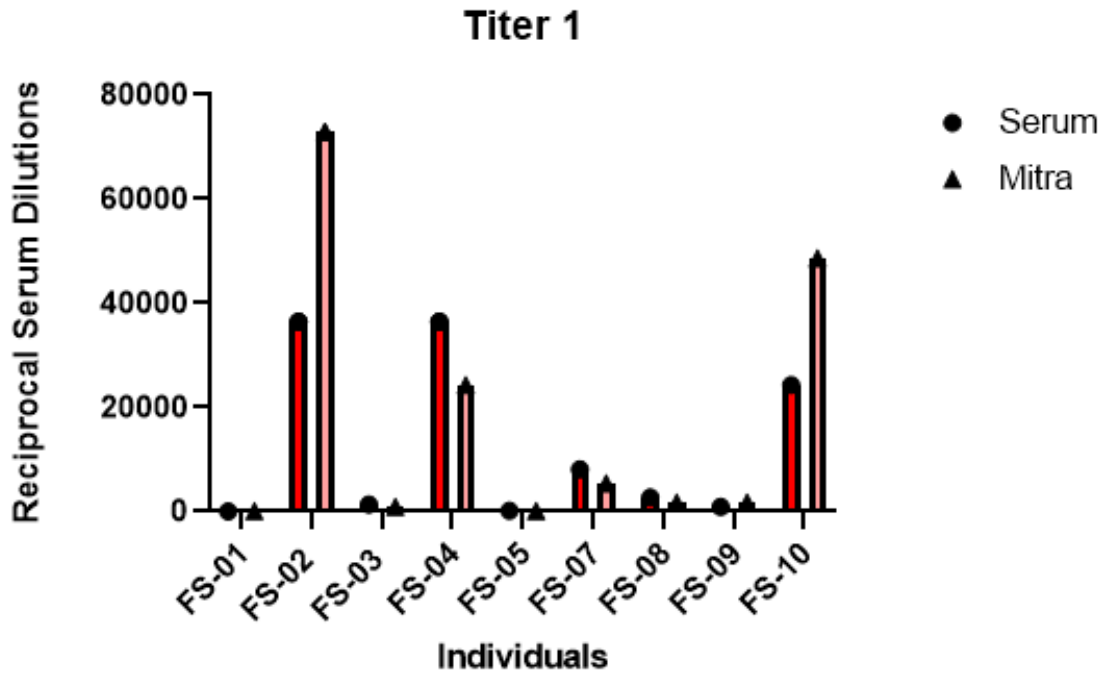


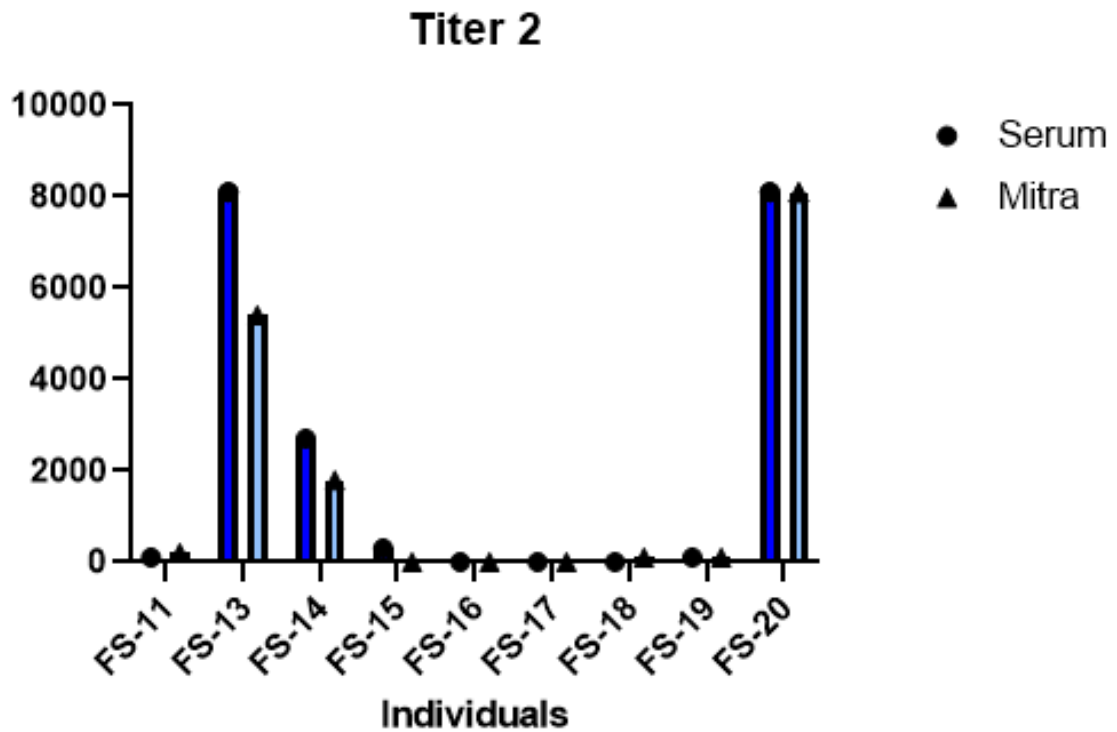
Figure 1C: Reciprocal Serum Dilutions 3 of 3.



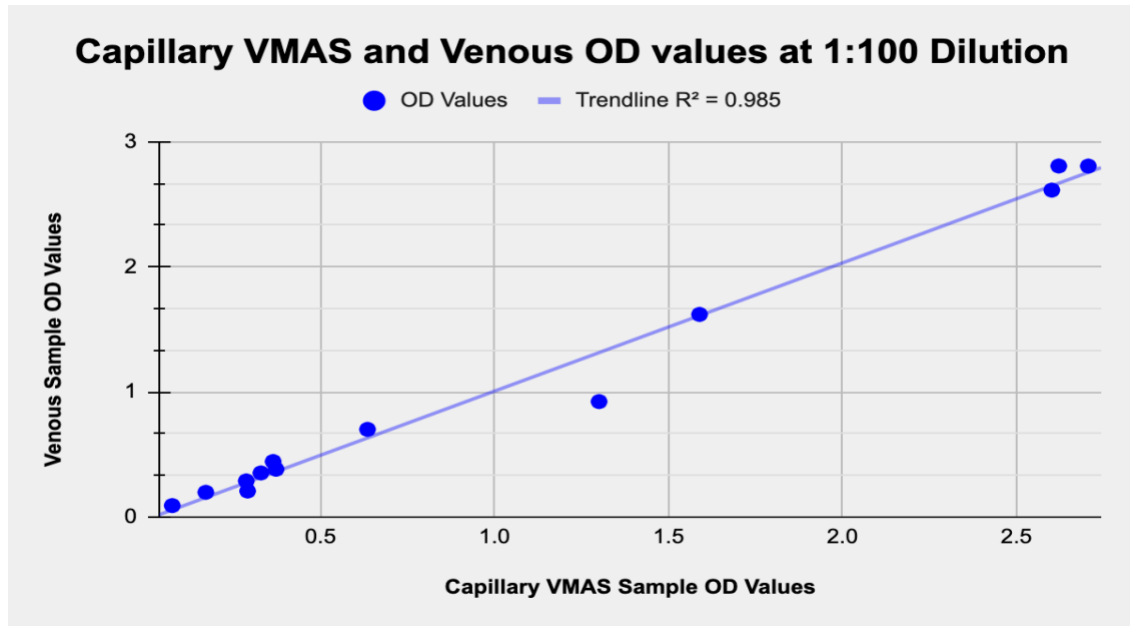
**Figure 2: Anti-spike RBD standard absorbance curves.** For every ELISA (E35, E36, E44, E45, and E48) standard curves were produced for each plate at a concentration of 25 ul/ml. Using the standard curve, you can predict the serum IgG concentrations in an unknown sample and it shows you the maximum OD value for any given assay. A new standard curve was produced for each assay to ensure reliable data interpretation.



**Figure 3A: Positive Control Reciprocal Serum Dilutions.** All positive control individuals (FS-01 thru FS-10) were plotted with their corresponding reciprocal serum dilutions. Paired serum is represented as circles and capillary VAMS represented with triangles. FS-06 omitted due to improper capillary VAMS sampling. FS-02, FS-04, FS-07, and FS-10 showed very high concentrations of anti-spike IgG in serum, but all subjects in the positive control group met their assay positivity threshold.



**Figure 3B: Negative Control Reciprocal Serum Dilutions.** All negative control individuals (FS-10 thru FS-20) were plotted with their corresponding reciprocal serum dilutions. Paired serum is represented as circles and capillary VAMS represented with triangles. FS-12 omitted due to improper capillary VAMS sampling. While the negative control group's reciprocal serum dilutions are much lower than those of the positive control group, FS-13, FS-14, and FS-20 met the positivity threshold for their given ELISA.



**Figure 4: Correlation of Positive Sample OD Values at a 1:100 Dilution.** To determine if capillary VAMS and venous serum anti-spike IgG concentrations correlate, we can take the average of the OD duplicates for each positive sample at a given dilution factor and plot them. OD values directly correlate with anti-spike IgG serum concentration levels. A strong positive correlation between the two testing methods is shown ( $R=0.985$ ).



Capillary VAMS Storage Times			
Sample	Date of Collection	Date of Assay	# of Days in Storage
FS-01	1/27/2021	3/3/2021	35
FS-02	1/21/2021	3/3/2021	41
FS-03	1/21/2021	3/3/2021	41
FS-04	1/27/2021	3/3/2021	35
FS-05	1/27/2021	3/4/2021	36
FS-07	2/9/2021	4/27/2021	77
FS-08	1/28/2021	4/27/2021	89
FS-09	2/15/2021	4/27/2021	71
FS-10	2/2/2021	4/27/2021	84
FS-11	2/2/2021	4/27/2021	84
FS-13	1/28/2021	5/4/2021	96
FS-14	2/1/2021	5/4/2021	92
FS-15	2/1/2021	5/4/2021	92
FS-16	2/1/2021	5/4/2021	92
FS-17	2/1/2021	5/4/2021	92
FS-18	2/1/2021	6/7/2021	126
FS-19	2/1/2021	6/7/2021	126
FS-20	2/2/2021	6/7/2021	125

**Table 1: Capillary VAMS pre-hydration storage times.** For each test subject, the date of collection represents the date the University of Kentucky’s Human Immune Monitoring Lab received the capillary samples from the Center for Clinical and Translational Science and the date of hydration represents the day they were hydrated in 1X PBS.

## CHAPTER 4. DISCUSSION

Especially in the early phases of a pandemic, it is critical to understand disease seroprevalence within a population to better determine disease control methods and stop geographical spread. Our results demonstrate that there is a very strong positive correlation between venous blood collection and Mitra home sampling for the detection of anti-spike IgGs. During the course of our study, other labs in the U.S. were concurrently testing capillary blood sampling to determine if this method would be acceptable for managing and monitoring the rise of future diseases. All of these groups showed similar results between the two sampling methods<sup>23,24,25</sup>. Adding to this research, our lab further demonstrated serum antibody correlation of capillary VAMS and conventional venous sampling methods using a highly-specific and quantifiable SARS CoV2 IgG enzyme-linked immunosorbent assay (Figure 4).

Due to limited serosurveillance protocols and improper testing methods at the start of the COVID-19 pandemic, many asymptomatic cases went undetected, causing the seroprevalence rates to be much higher than officials were estimating<sup>27</sup>. Subjects from our negative control group, FS-13, FS-14, and FS-20, had anti-spike IgG serum concentrations that were above the positivity threshold of the ELISA, implicating that they could have been asymptomatic carriers of SARS CoV2, as they had no known symptoms or positive test prior to the study. Using Figure 3B for reference, the three sero-positive subjects' endpoint titers were much lower than the positive subjects in Figure 3A. Even though they met the positivity threshold of the ELISA, their anti-spike IgG serum concentrations were still very low, meaning that if they were an asymptomatic case of SARS CoV2, their immune response was not robust enough to produce high

concentrations of serum anti-spike IgGs. In Figure 3A, endpoint titers of IgGs were much higher, meaning their immune response during an active infection was more profound and produced high levels of anti-spike IgG. While this assay has a high sensitivity and accuracy for detecting spike-specific IgGs in serum, we cannot conclude that those who meet the positivity threshold or a certain concentration threshold will have protective immunity toward a future SARS CoV2 infection. IgG viral neutralization effects vary by individual based on the quantity and time of SARS CoV2 exposure and their body's overall immune response<sup>28</sup>. We can assume that as a patient's serum anti-spike IgG concentration increases, they would have a better ability to neutralize SARS CoV2, but the neutralizing effects of anti-spike IgG have been shown to decline rapidly 5-8 weeks post-infection, but can remain detectable for up to 8 months<sup>29</sup>.

Although not originally in the study design, we also demonstrated antibody stability for up to 126 days at ambient temperature when dehydrated on Neoteryx's VAMS collection swab. In a publication by the National Institutes of Health studying capillary VAMS antibody stability, a research group showed 94% stability up to 21 days at room temperature before placing the samples at -20°C<sup>24</sup>. Our study further adds to these findings and increases the utility of capillary VAMS collection kits due to not needing any special storage or shipping protocols.

Using this minimally invasive and quick method of testing will allow kits to be sent in large numbers to people around the world, including rural and remote locations, allow for an exponential decrease in sample processing time, and reduce the cost associated with venous sampling and testing. Our highly-sensitive and quantitative SARS CoV2 ELISA protocol allows, with minor modifications, for use with virtually any viral

infection arising in the future. In combination with mail-in blood sampling kits, the way we monitor and control diseases within a population will forever change.

## REFERENCES

1. World Health Organization. (n.d.). *Who coronavirus (COVID-19) dashboard*. World Health Organization. Retrieved September 1, 2022, from <https://covid19.who.int/>
2. *American pandemic preparedness: Transforming our capabilities - white house*. (n.d.). Retrieved September 3, 2022, from <https://www.whitehouse.gov/wp-content/uploads/2021/09/American-Pandemic-Preparedness-Transforming-Our-Capabilities-Final-For-Web.pdf?page=29>
3. Kelly-Cirino, C. D., Nkengasong, J., Kettler, H., Tongio, I., Gay-Andrieu, F., Escadafal, C., Piot, P., Peeling, R. W., Gadde, R., & Boehme, C. (2019). Importance of diagnostics in epidemic and pandemic preparedness. *BMJ global health*, 4(Suppl 2), e001179. <https://doi.org/10.1136/bmjgh-2018-001179>
4. Bchetnia, M., Girard, C., Duchaine, C., & Laprise, C. (2020). The outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A review of the current global status. *Journal of infection and public health*, 13(11), 1601–1610. <https://doi.org/10.1016/j.jiph.2020.07.011>
5. Muralidar, S., Ambi, S. V., Sekaran, S., & Krishnan, U. M. (2020). The emergence of COVID-19 as a global pandemic: Understanding the epidemiology, immune response and potential therapeutic targets of SARS-CoV-2. *Biochimie*, 179, 85–100. <https://doi.org/10.1016/j.biochi.2020.09.018>
6. Wartecki A, Rzymiski P. On the Coronaviruses and Their Associations with the Aquatic Environment and Wastewater. *Water*. 2020; 12(6):1598. <https://doi.org/10.3390/w12061598>
7. Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends in immunology*, 41(12), 1100–1115. <https://doi.org/10.1016/j.it.2020.10.004>
8. Shirbhate, E., Pandey, J., Patel, V. K., Kamal, M., Jawaid, T., Gorain, B., Kesharwani, P., & Rajak, H. (2021). Understanding the role of ACE-2 receptor in pathogenesis of COVID-19 disease: a potential approach for therapeutic intervention. *Pharmacological reports : PR*, 73(6), 1539–1550. <https://doi.org/10.1007/s43440-021-00303-6>
9. Rauti, R., Shahoha, M., Leichtmann-Bardoogo, Y., Nasser, R., Paz, E., Tamir, R., Miller, V., Babich, T., Shaked, K., Ehrlich, A., Ioannidis, K., Nahmias, Y., Sharan, R., Ashery, U., & Maoz, B. M. (2021). Effect of SARS-CoV-2 proteins on vascular permeability. *eLife*, 10, e69314. <https://doi.org/10.7554/eLife.69314>
10. Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 Cytokine Storm; What We Know So Far. *Frontiers in immunology*, 11, 1446. <https://doi.org/10.3389/fimmu.2020.01446>
11. Mayo Foundation for Medical Education and Research. (n.d.). *History of covid-19: Outbreaks and vaccine timeline*. Mayo Clinic. Retrieved September 5, 2022, from <https://www.mayoclinic.org/coronavirus-covid-19/history-disease-outbreaks-vaccine-timeline/covid-19>
12. Levin, J., Bradshaw, M. Determinants of COVID-19 skepticism and SARS-CoV-2 vaccine hesitancy: findings from a national population survey of U.S.

- adults. *BMC Public Health* 22, 1047 (2022). <https://doi.org/10.1186/s12889-022-13477-2>
13. Mascellino, M. T., Di Timoteo, F., De Angelis, M., & Oliva, A. (2021). Overview of the Main Anti-SARS-CoV-2 Vaccines: Mechanism of Action, Efficacy and Safety. *Infection and drug resistance*, 14, 3459–3476. <https://doi.org/10.2147/IDR.S315727>
14. Hou, X., Zaks, T., Langer, R. *et al.* Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* 6, 1078–1094 (2021). <https://doi.org/10.1038/s41578-021-00358-0>
15. Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The major histocompatibility complex and its functions. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK27156/>
- 16 Gulati, P. (2009), Janeway's Immunobiology, 7th Edition by Kenneth Murphy, Paul Travers, and Mark Walport. *Biochem. Mol. Biol. Educ.*, 37: 134-134. <https://doi.org/10.1002/bmb.20272>
- 17 Dang, V. D., Hilgenberg, E., Ries, S., Shen, P., & Fillatreau, S. (2014). From the regulatory functions of B cells to the identification of cytokine-producing plasma cell subsets. *Current opinion in immunology*, 28, 77–83. <https://doi.org/10.1016/j.coi.2014.02.009>
18. Schroeder, H. W., Jr, & Cavacini, L. (2010). Structure and function of immunoglobulins. *The Journal of allergy and clinical immunology*, 125(2 Suppl 2), S41–S52. <https://doi.org/10.1016/j.jaci.2009.09.046>
19. Alhajj M, Farhana A. Enzyme Linked Immunosorbent Assay. [Updated 2022 Feb 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK555922/>
20. Arya, S. K., & Estrela, P. (2018). Recent Advances in Enhancement Strategies for Electrochemical ELISA-Based Immunoassays for Cancer Biomarker Detection. *Sensors (Basel, Switzerland)*, 18(7), 2010. <https://doi.org/10.3390/s18072010>
21. Kalish, H., Klumpp-Thomas, C., Hunsberger, S., Baus, H. A., Fay, M. P., Siripong, N., Wang, J., Hicks, J., Mehalko, J., Travers, J., Drew, M., Pauly, K., Spathies, J., Ngo, T., Adusei, K. M., Karkanitsa, M., Croker, J. A., Li, Y., Graubard, B. I., ... Sadtler, K. (2021, January 1). *Mapping a pandemic: SARS-COV-2 seropositivity in the United States*. medRxiv. Retrieved October 11, 2022, from <https://www.medrxiv.org/content/10.1101/2021.01.27.21250570v1.full-text>
22. Kumar, M. S., Bhatnagar, T., Manickam, P., Kumar, V. S., Rade, K., Shah, N., Kant, S., Babu, G. R., Zodpey, S., Girish Kumar, C. P., Vivian Thangaraj, J. W., Chatterjee, P., Kanungo, S., Pandey, R. M., Murhekar, M., Singh, S. K., Sarkar, S., Mulyi, J. P., Gangakhedkar, R. R., & Reddy, D. (2020). National sero-surveillance to monitor the trend of SARS-CoV-2 infection transmission in India: Protocol for community-based surveillance. *The Indian journal of medical research*, 151(5), 419–423. [https://doi.org/10.4103/ijmr.IJMR\\_1818\\_20](https://doi.org/10.4103/ijmr.IJMR_1818_20)
23. Brown, L., Byrne, R. L., Fraser, A., Owen, S. I., Cubas-Atienzar, A. I., Williams, C. T., Kay, G. A., Cuevas, L. E., Fitchett, J., Fletcher, T., Garrod, G., Kontogianni, K., Krishna, S., Menzies, S., Planche, T., Sainter, C., Staines, H. M., Turtle, L., & Adams, E. R. (2021). Self-sampling of capillary blood for SARS-

- CoV-2 serology. *Scientific reports*, 11(1), 7754. <https://doi.org/10.1038/s41598-021-86008-5>
24. Wang, J., Li, D., Wiltse, A., Emo, J., Hilchey, S. P., & Zand, M. S. (2019). Application of volumetric absorptive microsampling (VAMS) to measure multidimensional anti-influenza IgG antibodies by the mPlex-Flu assay. *Journal of clinical and translational science*, 3(6), 332–343. <https://doi.org/10.1017/cts.2019.410>
25. Žak, M. M., Stock, A., Stadlbauer, D., Zhang, W., Cummings, K., Marsiglia, W., Zargarov, A., Amanat, F., Tamayo, M., Cordon-Cardo, C., Krammer, F., & Mendu, D. R. (2021). Development and characterization of a quantitative ELISA to detect anti-SARS-CoV-2 spike antibodies. *Heliyon*, 7(12), e08444. <https://doi.org/10.1016/j.heliyon.2021.e08444>
26. Vai, S., Cavallo, R., Angeretti, A., Ferrara, B., Bongiasca, G., Voglino, G., & Merlino, C. (1987). Stabilità degli anticorpi specifici nel sangue essiccato su carta da filtro [Stability of specific antibodies in blood collected on filter paper disks]. *Giornale di batteriologia, virologia ed immunologia*, 80(1-12), 253–261.
27. Brown TS, Walensky RP. Serosurveillance and the COVID-19 Epidemic in the US: Undetected, Uncertain, and Out of Control. *JAMA*. 2020;324(8):749–751. doi:10.1001/jama.2020.14017
28. Maeda, K., Higashi-Kuwata, N., Kinoshita, N. *et al.* Neutralization of SARS-CoV-2 with IgG from COVID-19-convalescent plasma. *Sci Rep* 11, 5563 (2021). <https://doi.org/10.1038/s41598-021-84733-5>
29. Dispinseri, S., Secchi, M., Pirillo, M.F. *et al.* Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. *Nat Commun* 12, 2670 (2021). <https://doi.org/10.1038/s41467-021-22958-8>

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