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The effect of storage conditions on the preanalytical stability of Anti-SARS-CoV-2 antibodies in whole blood samples

Degree Project in Medicine

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Abstract

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Key Words: SARS-CoV-2, COVID-19, preanalytical stability, antibodies

Background: To combat the ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), mass-scale antibody testing is occurring. It is important that test results are accurate, yet varying preanalytical storage conditions could potentially influence antibody stability, resulting in false titer values.

Aim: This study primarily aims to investigate the effects of the preanalytical storage conditions of time, temperature, and collection tube type on Anti-SARS-CoV-2 antibody stability in order to allow for accurate interpretation of measured titer values. Additionally, it aims to determine if there is a correlation between titers and disease severity.

Method: Participants with previously confirmed or suspected COVID-19 completed a questionnaire regarding their symptoms. An initial T0 blood sample was collected and analyzed. Additional samples were collected in two pairs of serum and ethylenediaminetetraacetic acid (EDTA) tubes and stored in either room temperature or 4°C. Aliquots were removed and analyzed on days 1, 3, and 5. Resulting titer values were analyzed using Spearman's correlation and Wilcoxon signed-rank test.

Results: Fifteen participants were included. A slight titer decrease over time was observed in both serum and EDTA groups but was only significant in the EDTA group (room temperature p=0.050, 4°C p=0.018). The average decrease in the EDTA groups was 5.0AU/mL in room temperature and 4.5 AU/mL in 4°C. A significant correlation between T1, T3, T5, and T0 was observed in all groups with high r-values ranging from 0.883 – 0.995. No correlation was found between T0 titers and disease severity.

Conclusion: Titers in both serum and EDTA groups remained stable over time with no dramatic decreases regardless of storage conditions and can therefore be accurately interpreted. The slight antibody instability observed in the EDTA groups was minimal and considered clinically irrelevant but more research needs to be conducted to further investigate the results.

Abbreviations

WHO	World Health Organization
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019
RT-PCR	Reverse transcription polymerase chain reaction
NAb	Neutralizing antibody
IgM/G	Immunoglobulin M/G
VGR	Västra Götaland Region
EDTA	Ethylenediaminetetraacetic acid
AU/mL	Arbitrary units per milliliter
RT	Room temperature

1. Introduction

In December of 2019, the World Health Organization (WHO) was made aware of a cluster of cases of “pneumonia of unknown cause” in Wuhan, China [1]. The responsible pathogen, later to be identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread shortly thereafter across the globe, giving rise to the coronavirus disease (COVID-19) pandemic, as declared by the WHO on March 11, 2020 [1, 2]. At the time of writing (December 11, 2020), 9 months after the declaration of the pandemic, COVID-19 has led to over 1,500,000 deaths worldwide, with a total of confirmed cases of over 68,000,000 [3]. The current situation has yielded a broad field of unknowns to which the scientific community has been forced to quickly respond, spurring an intensive research effort globally in order to better understand and combat the pandemic.

Disclaimer: Seeing as the SARS-CoV-2 virus is a new pathogen to humans, all aspects regarding the resulting disease have been previously unknown. An extensive amount of research is therefore being conducted worldwide in order to find out more about how to curb its spread along with how to treat infected individuals. As new information is discovered, scientific articles are being produced at staggering speeds, leading to knowledge about COVID-19 continually updating itself. A direct result in regards to this research project is that information learned at the beginning of the project in August 2020 may have become outdated by the project’s completion in December 2020. In order to provide accurate facts while simultaneously allowing time for analysis and writing, this paper will not be updated with new research or current statistics pertaining to COVID-19 after December 11, 2020.

2. Background

2.1 Testing

The diagnostics of COVID-19 are currently based on three different methods: reverse transcription polymerase chain reaction (RT-PCR), serological determination of viral antigens, and evidence of antibodies against different parts of the SARS-CoV-2 virus [4]. Patients with active infections are diagnosed via RT-PCR [5, 6] and antigen testing, the former method being more common due to its higher sensitivity [7]. However, in November, the European Centre for Disease Prevention and Control did release a report highlighting the benefits of rapid antigen testing in situations where capacity for RT-PCR testing is limited [8]. As for convalescent or recovered patients who are virus-free with negative RT-PCR results, recent studies have confirmed that post-infection serological testing of antibody titers can be a useful, alternative, diagnostic method [9-11], a conclusion shared by the WHO [12, 13]. A rapid and correct diagnosis can then help health care professionals with proper clinical management of the individual and provision of efficient medical care.

Following a SARS-CoV-2 infection, humans can produce different types of antibodies: antibodies that bind to SARS-CoV-2 particles but have no neutralization capacity and neutralizing antibodies (NAbs). Detection of NAbs is considered the gold standard since they inhibit virus spread and protect against re-infection. Studies have been able to identify NAbs against SARS-CoV-2 [14, 15], including in patients who had tested negative using standard methods [16], and have also shown a correlation between NAbs and binding antibodies with NAb detection occurring 11-15 days post-onset [17]. Several tests can be used to detect NAbs but they take several days to get results [18], limiting their diagnostic usefulness and rendering the much quicker assays for binding antibodies as the current

standard [19, 20]. As for binding antibody response, one particular study showed immunoglobulin M (IgM) detection occurring an average of 5 days after symptom onset and immunoglobulin G (IgG) detection occurring after an average of 14 days [21]. It remains unclear, however, what a positive antibody test result would mean for an individual in practice, as there is little evidence supporting total immunity after recovery [12, 22], and a positive test shouldn't be equated with total protection against re-infection. A few recent studies did see a correlation between severity of symptoms and IgG antibody response, suggesting that individuals with a more severe clinical course may have a higher likelihood of immunity [23, 24]. However, other studies have demonstrated that measured antibody levels decrease significantly in the weeks following an infection [25, 26]. These concerns regarding such variation in individual responses have been highlighted within the scientific community, specifically over-interpretation of seroprevalence and immunity [22, 27]. Additionally, as more is learned about the SARS-CoV-2 virus and human immune response, a correlation has been shown to exist between antibody response and cell-mediated response through T-cells and memory B-cells [28, 29] which further complicates understanding of the role of antibodies. Both diagnostic and immunity potential can be found in post-infection antibodies, emphasizing the importance of accurate test results, but it should also be taken into account that immune responses are highly individual and it is still too early to know what the long-term immunological effects of COVID-19 will be.

2.2 Prevalence Tracking

Antibody testing may be able to assist in tracking COVID-19 prevalence and transmission, and has the potential of contributing on a larger, more concrete scale in epidemiological studies across the globe [30] to increase knowledge about the disease. One

factor which makes the process of identifying cases more difficult is that COVID-19 is a disease which presents with a variety of different symptoms, often mimicking those of other common illnesses [31-35]. There is also a large variation in severity of the disease, with cases ranging from asymptomatic to requiring time in the intensive care unit [36, 37]. Those with mild to moderate symptoms seldom require medical care and can recover at home on their own. Many international health organizations and governments, in order to prevent hospitals from overrunning, have therefore launched large informational campaigns, encouraging people to self-isolate if they experience any of the major COVID-19 symptoms such as fever or cough [38-40]. In Sweden, the list of reported symptoms requiring self-quarantine includes more vague symptoms as well, such as sore throat, runny nose, headache, nausea, and muscle and joint pain [41]. This variation in symptoms and the existence of so many asymptomatic cases has led to a state of ambiguity regarding the true prevalence of COVID-19, emphasizing the importance of having accurate data to better combat the pandemic and implement an effective long-term response.

2.3 The Current Situation and its Challenges

2.3.1 Testing Rate

An important aspect requiring particular consideration is the sheer amount of testing being done nationally. In June 2020, the Swedish government and the country's 21 regions came to an agreement to increase national testing for COVID-19, a strategy including both RT-PCR and antibody testing, in order to guide Sweden's pandemic response and prioritize testing of certain groups [42, 43]. Initially, there was an order of importance for antibody testing to prioritize patients and health care workers [44] but in October 2020, the Västra Götaland Region (VGR) expanded testing possibilities to include the general public

[45]. As of December 11, 2020, a total of over 32,000 antibody tests are being taken per week, nationally [46]. In order for mass testing to be implemented effectively, tests have to be analyzed quickly. A problem that can then occur is the use of rapid diagnostic tests which can vary in accuracy [47], an issue the WHO is attempting to address by developing a standardized serology assay for global use [30]. In spite of this, countries still vary on which methods they use. In Sweden's laboratories, a total of 7 antibody tests are currently being used and evaluated, each of which require different platforms and analyzers [48]. Titer values are therefore calculated and expressed differently depending on which platform is used, further highlighting the importance of relying on antibody stability to avoid misleading results. As the advantages and limitations of mass-scale antibody testing are still being discovered, considering the fact that such an increased rate of testing is being conducted at such high speeds, it is important that the results are as accurate as possible with minimal opportunities for error.

2.3.2 Logistics

Another factor which could potentially affect test accuracy is the conditions the blood samples are exposed to prior to test analysis. This problem, observed in Sweden as well as in other countries, is particularly relevant since COVID-19 antibody tests are only analyzed in a few laboratories across the country. These laboratories are usually found at the university hospitals which, apart from the large work burden, may also have limited working hours, preventing the immediate analysis of all incoming blood samples due to extended transport times and limited capacity. These preanalytical obstacles can lead to blood samples being transported over great distances and stored for hours or days at a time before processing occurs. In VGR, the designated laboratories for antibody testing are Sahlgrenska University

Hospital and the private laboratory network, Unilabs [49], both of which differ slightly in their testing protocols. As for Sahlgrenska University Hospital, transports in VGR attempt to deliver diagnostic tests as quickly as possible but despite their best attempts, 5% of the region's locations still take longer than 2 days to reach [50]. Serum is the standard test material and tests are recommended to be centrifuged and stored in a refrigerator prior to transport [51]. Unilabs also performs serum tests, stating that centrifugation is preferred but not mandatory and that tests can be sent in room temperature or in a refrigerator [52]. Additionally, while the standard response time is 1 – 4 days, tests can be stored in a refrigerator up to 3 days prior to transport [52]. It is mentioned that tests sent via post should arrive at the laboratory within two days [52], but Unilabs does also warn of the risk of sending tests too late in the week which could leaving them sitting at the post office over the weekend [53]. Sweden's national platform for information and services regarding healthcare, 1177 Vårdguiden, summarizes that in VGR, one should receive antibody test results within a week but that depends on where the test was taken [54]. While the increased strain on the laboratories due to mass-testing can very well have increased current processing time, under normal conditions, differing routines and human error can still lead to blood samples being affected by various storage conditions. Seeing as this is a general issue which has been identified in the past, several studies have already demonstrated the preanalytical stability of many different analytes, including antibodies [55, 56], in whole blood samples as well as plasma and serum samples after storage in various temperatures, for extended periods of time, and in different types of collection tubes [57, 58]. However, there have been no studies conducted on the preanalytical stability of specifically Anti-SARS-CoV-2 antibodies in

regards to any of the aforementioned factors, information which would greatly help one's ability to confidently rely on serological test results.

3. Aim

This study primarily aims to explore the effect of the storage conditions of time, temperature, and tube type on the stability of Anti-SARS-CoV-2 antibodies in blood samples prior to laboratory analysis. A secondary aim is to determine if there is a correlation between titer values and disease severity. The results will hopefully increase the understanding of Anti-SARS-CoV-2 antibody response to various storage conditions and allow for more accurate interpretation of titer values in COVID-19 antibody tests.

3.1 Hypothesis

The hypothesis is that Anti-SARS-CoV-2 antibodies will remain stable over time, regardless of studied storage conditions.

4. Materials and Methods

4.1 Participants

Participants (health care staff at Sahlgrenska University Hospital) were over 18 years of age and had either an RT-PCR confirmed SARS-CoV-2 infection in the previous 4-6 weeks or had symptoms after close contact with an individual with an RT-PCR confirmed SARS-CoV-2 infection. Participants were required to be symptom-free at the time of sample collection.

4.2 Procedure

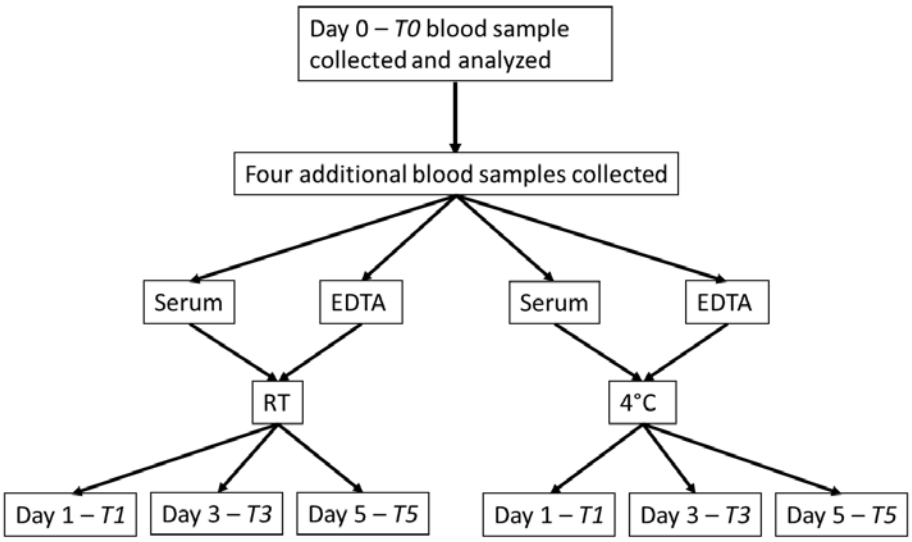

Figure 1: A schematic diagram illustrating the study design. *After initial blood samples were analyzed, additional samples were collected in both serum and ethylenediaminetetraacetic acid (EDTA) collection tubes and stored in either room temperature (RT) or 4°C. Aliquots from each tube were removed and sent for analysis on days 1, 3, and 5.*

4.2.1 Sample Collection

Venous blood samples were collected in the morning to avoid interference by postprandial hyperlipidemia. An initial 1mL sample was collected in a serum tube (VACUETTE® TUBE 2.5 ml CAT Serum Separator Clot Activator, Greiner Bio-One GmbH, Kremsmünster, Austria, ref. 454473) as specified by VGR's standard protocol [51] and sent immediately for analysis at Sahlgrenska University Hospital's Clinical Microbiology Laboratory to be used as a reference sample (T0). Four additional samples were then drawn into either two ethylenediaminetetraacetic acid (EDTA)-coated tubes (VACUETTE® TUBE 6 ml K2E K2EDTA, Greiner Bio-One GmbH, Kremsmünster, Austria, ref. 456243) or two tubes containing Serum Separator Clot Activator (VACUETTE® TUBE 9 ml CAT, Greiner

Bio-One GmbH, Kremsmünster, Austria, ref. 455009), all of which were then stored in room temperature (20-25°C) for 30 minutes to allow for clotting as per standard procedure [59].

4.2.2 Storage and Handling

After clotting, all sample tubes were stored horizontally and in the dark with one pair (EDTA and serum) being stored in a temperature of 4°C and the other pair being stored in room temperature (RT) between 20-25°C. After 1, 3, and 5 days, a 1.5mL aliquot from each tube (plasma from the EDTA tube and serum from the serum tube) was removed via a single-use syringe, centrifuged for 5 minutes at 2000xg, and then aliquoted again before finally being frozen at -80°C prior to analysis. The tubes were kept at a horizontal angle during the entire handling process and care was taken to prevent any air from entering the sample tubes. Tubes were gently handled so as not to cause any mechanical disturbances to the samples and prevent hemolysis.

4.2.3 Analysis Platform

IgG antibodies against SARS-CoV-2, were analyzed using two types of assays. To begin with, samples were screened using an Architect chemiluminescent microparticle immunoassay (Abbott Laboratories, USA) in order to detect the presence of antibodies against SARS-CoV-2 nucleocapsid-protein, a test which was considered positive if the value was ≥14 AU/mL. The samples that came up positive were then further analyzed with iFlash 1800 chemiluminescent immunoassay (YHLO Biotech Co., Shenzhen, China) in order to quantify the antibody titers detected, a process done by measuring IgG against SARS-CoV-2 viral spike-proteins or nucleocapsid-proteins with a value of ≥10 AU/mL considered positive.

4.3 Statistical Analysis

Results of antibody titers for each sample were collected and compared to the values from the T0 sample to see if storage temperature, time, or collection tube type had any effect on Anti-SARS-CoV-2 antibody titers. The programs used for data collection and analysis were Microsoft Excel and IBM SPSS Statistics. Spearman's correlation was used to study the relationship between participants' disease severity and initial T0 antibody titers. Descriptive statistics in the form of boxplots and scatterplots were carried out to visualize the between-group dynamics of the measured titer levels for each participant and within-group dynamics of titers in relation to participants' T0 values. In order to see if there was a significant change in titer value over time within groups depending on the aforementioned factors, Wilcoxon signed-rank test was used. A p-value of ≤ 0.05 was considered significant. The differences in titers over time was then compared between groups using the same test. As the independent variables were related, a 3 x 2 x 2 factorial repeated-measures analysis of variance would have been the best analysis of choice, seeing as this compensates for the risk of falsely significant values when doing multiple tests on the same data. However, lack of statistical competence prevented such a test from being conducted.

4.4 Questionnaire

A questionnaire was constructed with questions pertaining to participants' experienced symptoms during their SARS-CoV-2 infection (See Appendix). In conjunction with registering for the study, participants were asked to fill out the questionnaire in order to better assess the severity of their infections. The questionnaire included a list of common COVID-19 symptoms and participants were to answer yes or no in regards to whether or not

they had experienced said symptom. The questionnaire was constructed with aid from lists of common COVID-19 symptoms consolidated by the WHO [60] and Sweden's national public health authority, Folkhälsomyndigheten [61].

5. Ethics

The study was conducted in accordance with the Declaration of Helsinki's *Ethical Principles for Medical Research Involving Human Subjects* and has been reviewed and approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten) as part of a larger study, *COVID-19 (SARS-CoV-2 infektion) hos organtransplanterade (CORONA)*. The study was granted ethical approval on June 3, 2020 (reference #2020-02153) with an extension of the project allowing for this study having been approved on September 7, 2020 (reference #2020-04323). Permission to access patient charts in order to retrieve lab results was also granted by Per Lindnér, Department Head of Sahlgrenska University Hospital's Transplantation Center.

Ethical considerations taken into account consisted of confidentiality regarding participants' personal identification information and handling of their biological material. Participants were given an anonymized code number in conjunction with registering to allow for identification of blood samples. Samples were labeled with the code number to allow for proper handling. All information gathered from patient journals was then recorded and organized using the coded number. The only people with access to patient identification information and biological materials were the author and the thesis supervisor. Participants received written and oral information prior to registering and gave written, informed consent at the time of registration to sampling and accessing of identification information.

6. Results

6.1 Participants

Twenty participants registered for the study, 12 of whom were female (60%) and 8 male (40%), with the median age being 38 (range 21-58). Five out of the initial 20 registered participants had to be excluded from the study due to their initial T0 antibody titers being below detection limits. The remaining 15 participants went on to have additional tubes of blood drawn for continued use in the study. All of the participants had experienced what was classified as a mild case of COVID-19 and none had received any type of antiviral treatment.

6.2 Titers

A total of 13 aliquots (including T0) were analyzed per person. Antibody titers in these samples ranged from 14 – 138 AU/mL, with ten samples falling below the limit of detection of 14 and therefore given the value 7 as a placeholder during statistical analysis. The titer median of each group was calculated on days 0, 1, 3, and 5 (See Table 1) and plotted on boxplots in order to better visualize the titer dynamics (See Fig. 2). As can be seen in Figure 2, all four groups follow a downward trend with titers on day 5 being lower than those on day 0. The serum groups follow a stable decreasing trend while the EDTA groups follow a more varied course, experiencing an increase on day 3 in both RT and 4°C.

Table 1: The median titer values of each studied group calculated on days 0, 1, 3, and 5. *The range is in parentheses. RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

	SERUM RT		SERUM 4°C		EDTA RT		EDTA 4°C	
DAY 0 – T0	66	(15 – 117)	66	(15 – 117)	66	(15 – 117)	66	(15 – 117)
DAY 1 – T1	53	(7 – 115)	55	(7 – 118)	53	(7 – 119)	48	(7 – 117)
DAY 3 – T3	53	(7 – 120)	53	(7 – 118)	60	(7 – 138)	57	(7 – 132)
DAY 5 – T5	52	(7 – 120)	50	(7 – 118)	51	(19 – 82)	48	(19 – 88)

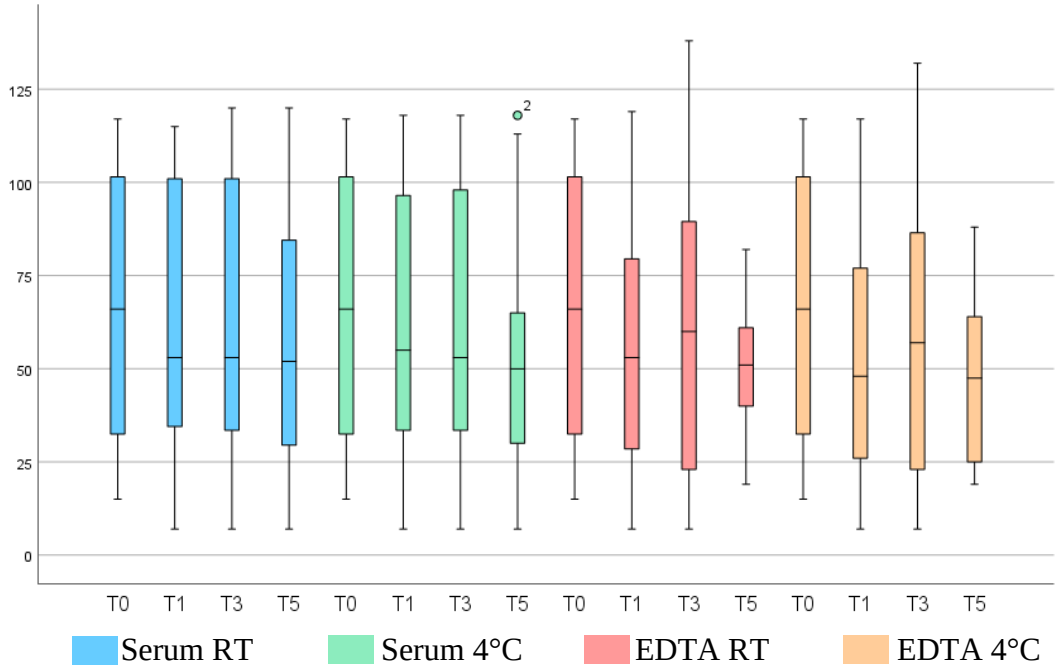

Figure 2: Boxplot illustrating the median titer values (including minimum and maximum values and interquartile range) of each studied group. *Values were calculated on days 0, 1, 3, and 5. Titer is on the y-axis and day is on the x-axis. RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

As displayed in Figure 3, the serum median ratios remained closer to 1.00 with the majority falling slightly under, while the EDTA median ratios were more varied with more ratios falling above 1.00 than in serum groups. The EDTA groups also had a larger range of ratios than the serum groups. When analyzing the relationship between T0 and T1, T3, T5 titers in each group, both serum and EDTA groups had a strong correlation although serum groups (r: 0.974 – 0.995) were slightly stronger than EDTA groups (r: 0.883 – 0.967). All correlations were significant. (See Table 2). This is further illustrated in Figure 4, with all

groups following a similar trend but the EDTA groups displaying a larger spread than the serum groups.

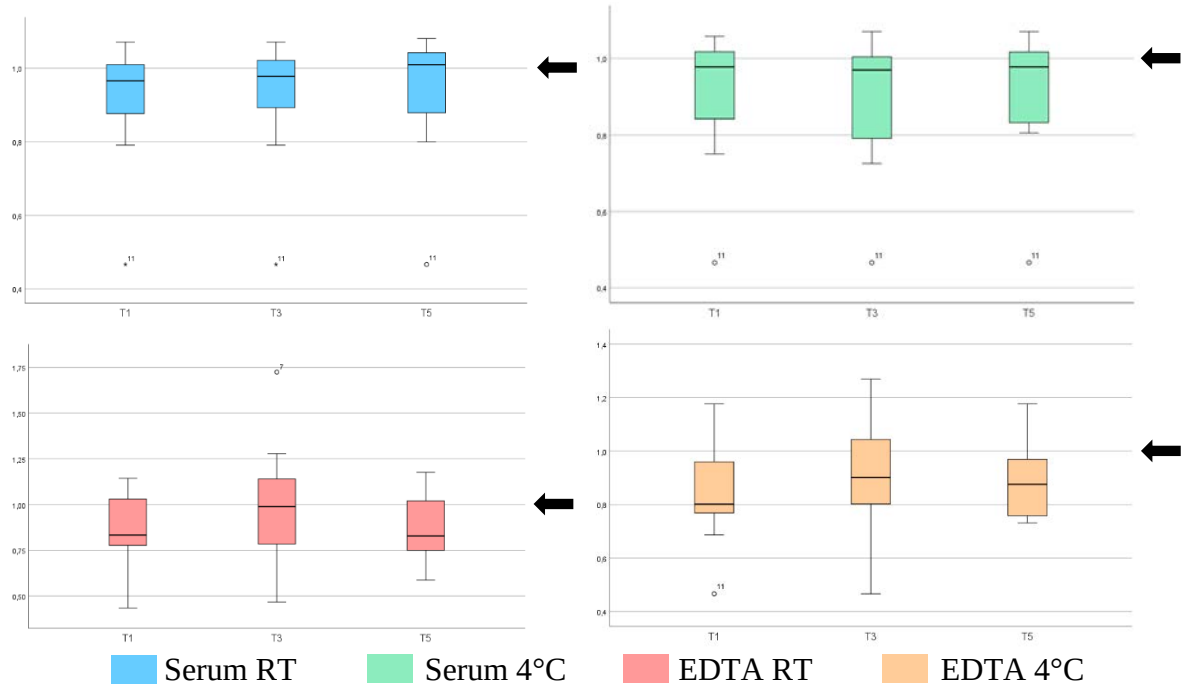

Figure 3: Boxplots illustrating the median ratio of T0 to T1, T3, T5 for each studied group. *The ratio of participants' antibody titer values to their respective T0 values is on the y-axis. Day is on the x-axis. The black arrow emphasizes the 1.00 line. RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

Table 2: The calculated median ratio, correlation coefficient (r), and confidence interval for T1, T3, T5 in each group compared to T0. *All correlation coefficients are significant. RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

VARIABLE COMPARED TO T0	MEDIAN RATIO	SPEARMAN'S CORRELATION (*R*)	CONFIDENCE INTERVAL
SERUM T1 RT	0.966	0.991	(0.860 – 1.000)
SERUM T3 RT	0.978	0.991	(0.860 – 1.000)
SERUM T5 RT	1.010	0.989	(0.860 – 1.000)
SERUM T1 4°C	0.978	0.993	(0.916 – 1.000)
SERUM T3 4°C	0.970	0.974	(0.847 – 1.000)
SERUM T5 4°C	0.978	0.995	(0.916 – 1.000)
EDTA T1 RT	0.833	0.893	(0.400 – 1.000)
EDTA T3 RT	0.990	0.883	(0.400 – 1.000)
EDTA T5 RT	0.828	0.929	(0.412 – 1.000)
EDTA T1 4°C	0.802	0.933	(0.586 – 1.000)
EDTA T3 4°C	0.902	0.967	(0.737 – 1.000)
EDTA T5 4°C	0.876	0.967	(0.737 – 1.000)

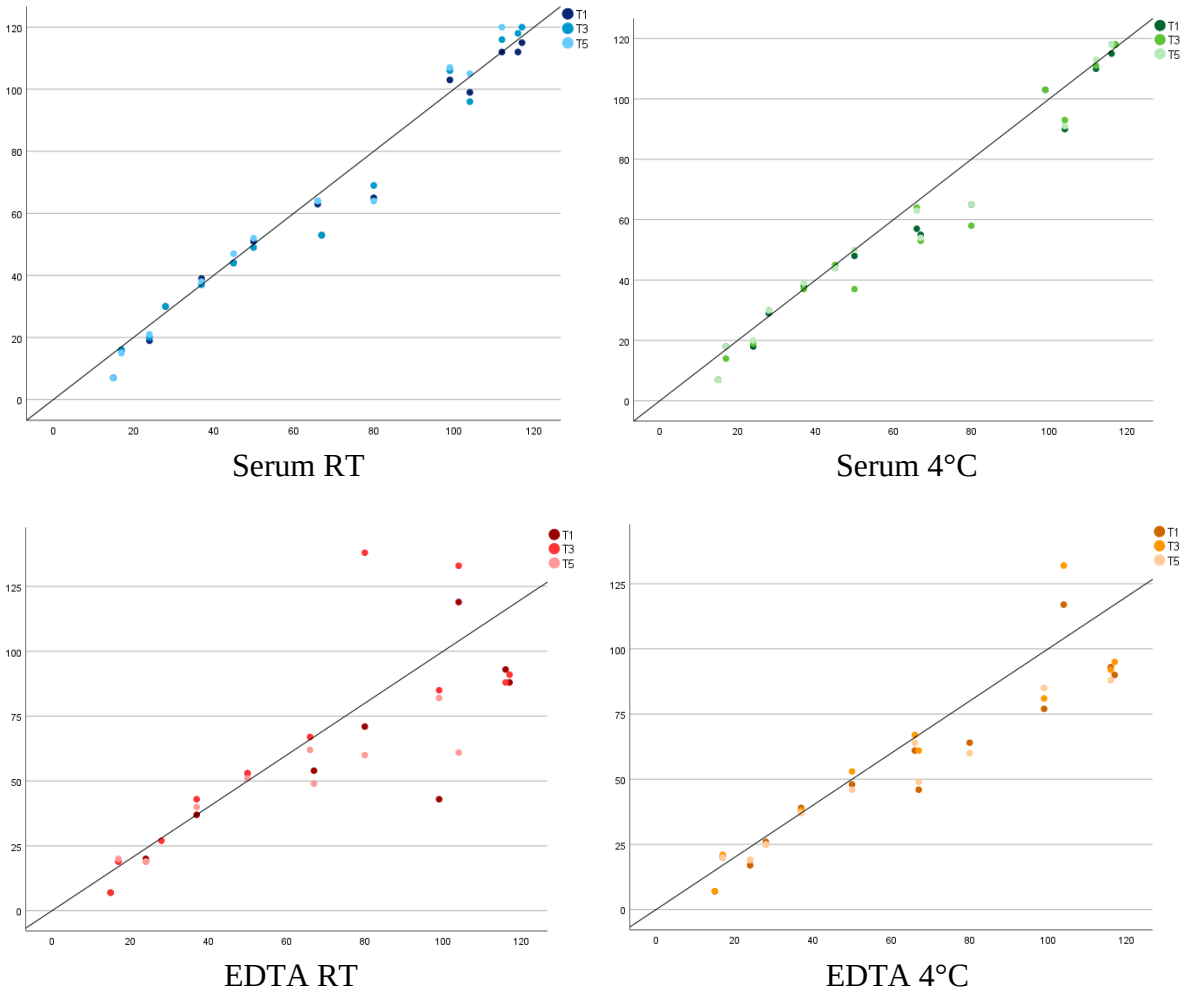

Figure 4: Scatterplots illustrating the correlation between T0 and T1, T3, T5 for each studied group. *The x-axis represents T0 titer values and the y-axis represents T1, T3, T5 titer values. The black line represents y=x. RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

As shown in Table 3, when T5 titers of each group were compared to the T0 titers, a significant decrease was found in the EDTA tubes stored in both RT (5.0 AU/mL) and 4°C (4.5 AU/mL). A significant change was also found when comparing T5 titers between groups in EDTA and serum tubes stored in 4°C (-4.0 AU/mL).

Table 3: The results of conducted statistical analyses comparing T0 to T5 in each group and T5 between groups. *The left column displays which groups were compared. The middle column displays the median difference in titers between each group (AU/mL). The calculated p-values for Wilcoxon signed-rank test are displayed in the right column with the significant values in bold (p≤0.05 is considered significant). RT = room temperature, EDTA = ethylenediaminetetraacetic acid.*

COMPARED GROUPS	MEDIAN	WILCOXON *P* -VALUE
T0 – SERUM T5 RT	-1.0	0.788
T0 – SERUM T5 4°C	1.0	0.115
T0 – EDTA T5 RT	5.0	**0.050**
T0 – EDTA T5 4°C	4.5	**0.018**
SERUM T5 RT – SERUM T5 4°C	-1.0	0.205
EDTA T5 RT – EDTA T5 4°C	0.0	0.581
SERUM T5 RT – EDTA T5 RT	-2.0	0.513
SERUM T5 4°C – EDTA T5 4°C	-4.0	**0.037**

6.3 Questionnaire

Eight participants failed to return a completed questionnaire and two of the returned questionnaires belonged to participants with negative T0 titers, thereby excluding 10 participants from statistical analysis. The average number of experienced symptoms was 7 (range 4 - 10). The participants' number of experienced symptoms was compared to their T0 antibody titer value using Spearman's correlation (See Fig. 5). There was no statistically significant correlation found between number of symptoms and initial titer value.

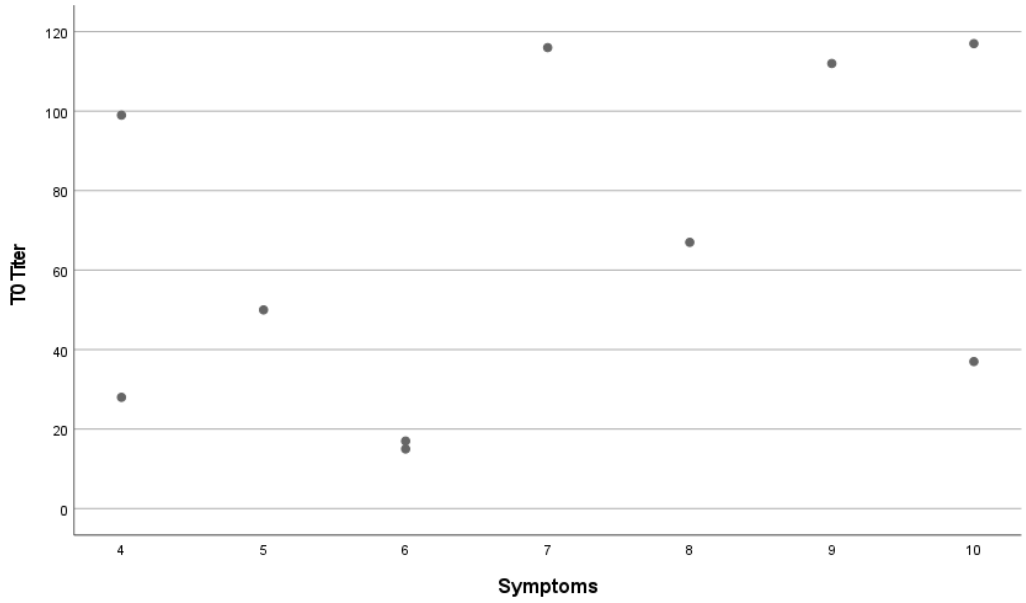

Figure 5: A scatterplot illustrating the number of experienced symptoms and T0 titers of each participant. *Participants with missing questionnaires or negative T0 titers have been removed (n=10). Correlation coefficient (r)=0.416, p-value=0.232.*

7. Discussion

7.1 Primary Findings

The hypothesis of this study was that the Anti-SARS-CoV-2 antibodies would remain stable, regardless of storage conditions, therefore allowing for accurate interpretation of titer values post-analysis. The results showed that titers remained stable in samples stored in serum tubes but decreased slightly in those stored in EDTA tubes, particularly when stored in 4°C. However, while this larger titer variation in the EDTA groups does warrant further exploration, there were no dramatic changes in titers over time in either group and the observed titer decreases were minimal. Furthermore, a decrease of 4 or 5 AU/mL in titer values that ranged from 14 to 138 AU/mL was considered too small, proportionally, to be deemed clinically relevant. Therefore, the results of this study imply that titer values

measured 1, 3, or 5 days post-collection after storage in various conditions are sufficiently stable compared to T0 titers to allow for accurate interpretation of measured titer values.

Many earlier studies have demonstrated antibody stability in various storage conditions as well. One study performed on 106 patients found no clinically significant change in antibody concentrations after storage in room temperature in serum or lithium-heparin tubes for 10 hours [55]. In another experiment conducted very similarly to this one, studying antibodies to 35 antigens (including many viruses) in 29 subjects, there was found to be no decrease in titers after storage over 6 days in both room temperature and 4°C in serum, EDTA, acid-citrate-dextrose, or lithium heparin vacutainers, concluding that there was no impact by processing delays, storage temperature, or vacutainer type on antibody stability [56]. Considering these past findings, it can be assumed that Anti-SARS-CoV-2 antibodies would behave similarly, an assumption seemingly supported by the results of this study.

While titers in all groups seemed to follow a slight downward trend, the EDTA groups had a more varied course and experienced an increase on day 3, the cause of which is unclear. One possible explanation is that of hemoconcentration, or water shifting into cells, a phenomenon that has been observed when studying analytes such as albumin and proteins [62, 63]. The amount of fluid in the sample tubes was constant but hemoconcentration could have potentially led to an interpreted increase in titers by the analyzer. The reason for this observation occurring in only the EDTA groups and not the serum groups is unknown and more research is needed to elucidate this finding.

The results in this study additionally showed no correlation between disease severity and initial antibody titers. However, this may have been due to the lack of completed questionnaires. In addition, all of the participants had what was classified as a mild or

moderate case of COVID-19. It may have been possible to see a correlation if the study had been larger and also included participants with severe infections. Several larger studies have delivered results contradictory to those in this study, namely a significant positive correlation between IgG antibody levels and symptom severity [23, 24, 64, 65]. A recent study conducted at Sahlgrenska University Hospital showed, again, a positive correlation with higher severity being related to higher IgG response after studying 47 patients classified as severe or mild cases based on requiring invasive mechanical ventilation or high-flow nasal oxygen and in-patient hospital care or not [16]. The study is especially significant seeing as it was conducted at the same location as this study and the exact same analysis process and platforms were used, suggesting that the results of this study didn't reach the same conclusions due to small size and lack of variation in disease severity.

Serum tubes are considered the standard for serological analysis of blood samples. EDTA tubes are standard for analysis of leukocytes including peripheral blood mononuclear cells such as B-cells and T-cells [66-68] while also allowing for antibody analysis. Given that both aspects of immune response toward SARS-CoV-2 are of great interest, it can be assumed that blood samples are occasionally collected in EDTA tubes instead of serum tubes to allow for studying of multiple analytes at once. The results of this study support the continued usage of serum tubes for Anti-SARS-CoV-2 antibody testing due to maintained antibody stability over 5 days of storage in both room temperature and 4°C. Regarding EDTA tubes, results reflect a greater instability than in serum tubes but a fairly high stability in general, suggesting that EDTA tubes can also be used without affecting titer accuracy. However, the effect of storage in EDTA tubes on antibody stability should be

further studied to investigate if the observed titer decreases in this study were true or can be attributed to as yet unidentified confounders.

7.2 Limitations

A substantial limitation of this study was the sample size. Although the initial number of 20 participants is generally considered the minimum for statistically significant results, once participants had to be excluded due to negative T0 titer values, 15 was too few to confidently rely on the statistical analyses. Another limitation was that several of the titer results were missing, one reason being human error and the other lack of sampling material. A larger study would have to be completed with more accurate statistical testing in order to give the results more power. An additional aspect to change would be including patients with a larger variety of COVID-19 severity, especially those with more severe cases. A correlation analysis might then have better mirrored real-life conditions and the results might have shown a different relationship between titers and severity. This study investigated storage over a 5-day time period but seeing as some situations could lead to a longer storage period, studying antibody stability over a more extended period of time would be of interest. Despite its faults, this study had a strong hypothesis and clear methodology, allowing for replications of the study in the future that will hopefully avoid the same obstacles and give more reliable results.

7.3 Further Research

As more is learned about human immune response to SARS-CoV-2 infections, it remains unclear how much of a role antibodies play compared to T-cells. In a study published in Sweden recently, memory T-cell responses were found in individuals both with and without the presence of antibodies, suggesting that a cell-mediated immune response might be

more determinant of protection against COVID-19 than seropositivity [69]. Since there is such uncertainty surrounding antibody testing with regards to protection from re-infection, it has been argued that antibody testing is not relevant for individual decision making at this time but can be useful as a public health tool [70]. The results of this study should therefore not be interpreted as a way to allow individuals to use their titer result to dictate their lifestyle choices, but rather to allow health care organizations and professionals to trust the accuracy of titers in order to improve their understanding of the disease response in humans while further research is conducted.

Despite the ambiguity of so many aspects surrounding Anti-SARS-CoV-2 antibodies, particularly their relevance to immunity, there are promising areas of research to which they can contribute. One such area is the development of a vaccine. One study analyzing NAb response in 59 patients found a direct positive correlation with disease severity but also a large variation in which specific NAbs were produced, highlighting the heterogeneity of NAb responses and therefore the need for a vaccine to protect people from future infection, particularly those with mild or asymptomatic cases [71]. Similarly, a large variation of NAbs in 175 recovered COVID-19 patients was found along with a correlation between titers and age but with some subjects failing to develop titers at all despite having a similar disease duration [17]. These findings on the diversity of antibody responses in individuals and how that affects immune response further support the notion that a positive antibody test should not be interpreted by an individual as automatic immunity, but can be useful in more large-scale projects such as the development of a vaccine based off population data.

Another area which is currently being studied is that of plasma therapy, a treatment in which seropositive plasma from convalescent individuals is transfused to those with a current SARS-CoV-2 infection in order to help them fight the virus. This has been demonstrated in several studies where critically ill patients treated with convalescent plasma showed an improvement in clinical status after treatment [72-75]. Some problems that could potentially arise with the implementation of using convalescent plasma as a treatment and prophylaxis large-scale have been brought to light, emphasizing such areas as donor criteria, recruitment, optimal dosing, distribution of plasma, and antibody testing [76]. Due to there being such focus on titers and what they mean for efficacy of plasma therapy, the results of this study or similar studies could provide beneficial information to further research on the subject.

8. Conclusion

The aim of this study was to not only provide more knowledge about Anti-SARS-CoV-2 antibodies in a time where little exists, but to also allow for accurate interpretation of antibody titer values, despite several important preanalytical variables that could potentially impact the results. The hope was to lead to more reliable data regarding COVID-19 prevalence as well as open the door to further research in the area of immune response to SARS-CoV-2. It was hypothesized that antibody titers would remain stable over time, regardless of storage temperature or tube type. Results show a greater tendency towards instability in antibodies in samples stored in EDTA tubes but an overall stability over time in samples from both serum tubes and EDTA tubes regardless of storage temperature, implying that titer values from samples stored for up to 5 days in both serum and EDTA tubes, room

temperature and 4°C can be interpreted as accurate. This study will hopefully encourage more research on the subject and serve as inspiration for similar studies on a larger scale, the results of which could further illuminate the findings in this study as well as provide valuable information in the development of a vaccine or use of plasma therapy. As stated by the WHO, "A complete understanding of the epidemiology and global risk posed by SARS-CoV-2 requires systematic serologic testing…By conducting surveys among different populations around the world, we can together understand the extent of the COVID-19 pandemic, which in turn will allow local, national, and international decision-makers to respond collectively to the pandemic [30]," a goal which has remained constant throughout this project and remains so even now.

Populärvetenskaplig Sammanfattning

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Påverkas nivån av COVID-antikroppar i blodprover av vissa förvaringsförhållanden?

Det har förmodligen inte undgått någon att vi befinner oss mitt i en pandemi. Länder över hela världen försöker bekämpa viruset SARS-CoV-2 (”severe acute respiratory syndrome coronavirus 2”), vilket orsakar sjukdomen “Coronavirus disease 2019”, mer känt som COVID-19. Det finns dock fortfarande många frågor som forskningen försöker besvara. Några viktiga frågor rör områden som innefattar virusets spridning och om en genomgången COVID-19 infektion gör dig immun mot återinfektion. Detta har lett till en dramatisk provtagningsökning globalt för att både individer, regeringar, och forskare ska kunna få information om smittspridning, sjukdomsfall, och immunitet.

De med pågående infektioner testas via svalgprov, medan de som har återhämtat sig kan ta ett blodprov för att mäta antikroppsnivåer. Vad gäller antikroppstester så har ett positivt testresultat bedömts av svenska Folkhälsomyndigheten att ge upp till 6 månaders immunitet. Längre än så vet vi dock inte hur immuniteten påverkas och det pågår oerhört mycket forskning kring ämnet. Så mycket testning som just nu pågår leder till att laboratorier jobbar under maximal kapacitet vilket potentiellt kan leda till att de blir överväldigade. I vissa fall kan det ta flera dagar innan blodprover analyseras och proverna förvaras under olika förhållanden under den tiden. För att säkerställa att antikroppstesternas resultat är så exakta som möjligt, ville vi studera om de uppmätta antikropparna speglade de sanna, originella antikroppsnivåerna, trots flera olika förvaringsförhållanden som potentiellt skulle kunna påverka antikropparna i blodproverna.

Vi tittade på effekterna av tid, förvaringstemperatur och blodrörstyp för att svara på denna fråga. COVID-19 antikroppstester (blodprover) togs på 15 personer där analys gjordes omedelbart. Sedan togs ytterligare prover som förvarades i två typer av rör (serum och ”ethylenediaminetetraacetic acid” härefter förkortat EDTA) och i två olika temperaturer (rumstemperatur och 4°C) i 5 dagar. Vi såg att nivån av antikroppar efter 5 dagar under dessa förhållanden förblev mycket nära dem från det första blodprovet, vilket antyder att tid, förvaringstemperatur, och rörtyp inte påverkar antikroppsnivåer och testresultaten mest troligt kan litas på. Vi såg en mycket liten minskning av antikroppar i de prover som förvarats i EDTA-rör, vilket inte är kliniskt relevant men betyder att fler studier bör göras på ämnet för att se om de kan återskapa samma resultat och se om detta var ett faktiskt resultat eller orsakat av en okänd faktor.

Dessa resultat betyder att vi med mest sannolikt kan lita på uppmätta antikroppsnivåer och testresultat så att de kan användas för att möjliggöra mer forskning om COVID-19-spridning och immunitet, data som förhoppningsvis kan användas för att skapa ett vaccin eller behandla sjukdomen på olika sätt. Ju mer vi kan lita på våra tester, desto mer kan vi förstå pandemin och bekämpa spridningen av viruset och medan mer forskning behöver göras är vår studie ett steg i rätt riktning.

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References

1. World Health Organization. *Timeline of WHO's response to COVID-19*. 2020 [cited 2020 25 Aug]; Available from: https://www.who.int/news-room/detail/29-06-2020-covidtimeline.
2. World Health Organization. *Naming the coronavirus disease (COVID-19) and the virus that causes it*. 2020 [cited 2020 25 Aug]; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it.
3. World Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard*. 2020 [cited 2020 11 Dec]; Available from: https://covid19.who.int/.
4. U.S. Food & Drug Administration. *Coronavirus Testing Basics*. 2020 [cited 2020 23 Sept]; Available from: https://www.fda.gov/consumers/consumer-updates/coronavirus-testing-basics.
5. Corman, V.M., et al., *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.* Euro Surveill, 2020. **25**(3).
6. European Centre for Disease Prevention and Control. *Diagnostic testing and screening for SARS-CoV-2*. 2020 [cited 2020 16 Sept]; Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/diagnostic-testing.
7. Mak, G.C., et al., *Evaluation of rapid antigen test for detection of SARS-CoV-2 virus.* Journal of clinical virology, 2020. **129**.
8. European Centre for Disease Prevention and Control. *Options for the use of rapid antigen tests for COVID-19 in the EU/EEA and the UK*. 2020 [cited 2020 24 Nov]; Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Options-use-of-rapid-antigen-tests-for-COVID-19_0.pdf.
9. Long, Q.X., et al., *Antibody responses to SARS-CoV-2 in patients with COVID-19.* Nat Med, 2020. **26**(6): p. 845-848.
10. Ni, L., et al., *Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals.* Immunity, 2020. **52**(6): p. 971-977.e3.

11. Okba, N.M.A., et al., *Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients.* Emerg Infect Dis, 2020. **26**(7): p. 1478-1488.
12. World Health Organization. *"Immunity passports" in the context of COVID-19*. 2020 [cited 2020 26 Aug]; Available from: https://www.who.int/news-room/commentaries/detail/immunity-passports-in-the-context-of-covid-19?gclid=Cj0KCQjwwOz6BRCgARIsAKEG4FXCGdF5N49Q4wmL_zO5Ujs_AvcMGK9O1nXG-GgtivThSXtMVcE_cZUaAm5WEALw_wcB.
13. World Health Organization. *Serology in the context of COVID-19*. 2020 [cited 2020 26 Aug]; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/serology-in-the-context-of-covid-19.
14. Wang, C., et al., *A human monoclonal antibody blocking SARS-CoV-2 infection.* Nature Communications, 2020. **11**(1): p. 2251.
15. Huang, A.T., et al., *A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity.* Nature communications, 2020. **11**(1): p. 4704.
16. Marklund, E., et al., *Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders.* PLOS ONE, 2020. **15**(10): p. e0241104.
17. Wu, F., et al., *Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications.* 2020.
18. Centers for Disease Control and Prevention. *Antibody Testing Interim Guidelines*. 2020 [cited 2020 24 Nov]; Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html#table1.
19. Perera, R.A., et al., *Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020.* Eurosurveillance, 2020. **25**(16): p. 2000421.
20. Muruato, A.E., et al., *A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation.* Nature Communications, 2020. **11**(1): p. 4059.
21. Guo, L., et al., *Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19).* Clin Infect Dis, 2020. **71**(15): p. 778-785.

22. Theel, E.S., et al., *The Role of Antibody Testing for SARS-CoV-2: Is There One?* J Clin Microbiol, 2020. **58**(8).
23. Reifer, J., et al., *SARS-CoV-2 IgG antibody responses in New York City*. Diagn Microbiol Infect Dis, 2020. **98**(3): p. 115128.
24. Young, B.E., et al., *Viral dynamics and immune correlates of COVID-19 disease severity.* Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2020.
25. Long, Q.X., et al., *Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections.* Nature Medicine, 2020. **26**(8): p. 1200-1204.
26. Liu, A., et al., *Antibody responses against SARS-CoV-2 in COVID-19 patients.* Journal of medical virology, 2020.
27. Gray, C.M., et al., *COVID-19 antibody testing: From hype to immunological reality.* South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde, 2020. **110**(9): p. 837.
28. European Centre for Disease Prevention and Control. *Immune responses and immunity to SARS-CoV-2*. 2020 [cited 2020 25 Sept]; Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/immune-responses.
29. Cox, R.J. and K.A. Brokstad, *Not just antibodies: B cells and T cells mediate immunity to COVID-19.* Nature Reviews Immunology, 2020.
30. World Health Organization. *"Solidarity II" global serologic study for COVID-19.* 2020 [cited 2020 28 Aug]; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-2-global-serologic-study-for-covid-19.
31. Carpenter, C.R., et al., *Diagnosing COVID-19 in the Emergency Department: A Scoping Review of Clinical Examinations, Laboratory Tests, Imaging Accuracy, and Biases.* Acad Emerg Med, 2020.
32. Lechien, J.R., et al., *Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019.* J Intern Med, 2020. **288**(3): p. 335-344.
33. World Health Organization. *Q&A: Influenza and COVID-19 - similarities and differences*. 2020 [cited 2020 29 Aug]; Available from:

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-similarities-and-differences-covid-19-and-influenza?gclid=Cj0KCQjwhIP6BRCMARIsALu9Lfn0a6wRYuYDsS_uKB9j5Mwy_ERp-k_pLv30I-Z172GIAGhtTHb_sZwaAkREEALw_wcB.

34. Centers for Disease Control and Prevention. *Symptoms of Coronavirus*. 2020 [cited 2020 26 Aug]; Available from: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html.
35. Pormohammad, A., et al., *Comparison of confirmed COVID-19 with SARS and MERS cases - Clinical characteristics, laboratory findings, radiographic signs and outcomes: A systematic review and meta-analysis.* Rev Med Virol, 2020. **30**(4): p. e2112.
36. European Centre for Disease Prevention and Control. *Clinical characteristics of COVID-19*. 2020 [cited 2020 29 Aug]; Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/clinical.
37. Li, J., et al., *Epidemiology of COVID-19: A systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes*. J Med Virol, 2020.
38. Centers for Disease Control and Prevention. *What to Do If You Are Sick*. 2020 [cited 2020 30 Aug]; Available from: https://www.cdc.gov/coronavirus/2019-ncov/if-you-are-sick/steps-when-sick.html.
39. Folkhälsomyndigheten. *Skydda dig och andra från smittspridning*. 2020 [cited 2020 30 Aug]; Available from: https://www.folkhalsomyndigheten.se/smittskydd-beredskap/utbrott/aktuella-utbrott/covid-19/skydda-dig-och-andra/#:~:text=Stanna%20hemma%20n%C3%A4r%20du%20%C3%A4r,bara%20k%C3%A4nner%20dig%20lite%20sjuk.
40. National Health Service. *When to self-isolate and what to do*. 2020 [cited 2020 25 Sept]; Available from: https://www.nhs.uk/conditions/coronavirus-covid-19/self-isolation-and-treatment/when-to-self-isolate-and-what-to-do/.
41. Folkhälsomyndigheten. *FAQ about COVID-19*. 2020 [cited 2020 27 Aug]; Available from: https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/communicable-disease-control/covid-19/?exp=68197#_68197.

42. Regeringen. *Bemyndigande att underteckna en överenskommelse mellan staten och Sveriges Kommuner och Regioner*. 2020 [cited 2020 30 Aug]; Available from: https://www.regeringen.se/49f19b/contentassets/7267e81035484338a41a152b14315d96/okad-nationell-testning-for-covid-19-2020-overenskommelse-mellan-staten-och-skr.pdf.
43. Folkhälsomyndigheten. *Nationell strategi för utökad provtagning och laboratorieanalys av covid-19*. 2020 [cited 2020 25 Sept]; Available from: https://www.folkhalsomyndigheten.se/contentassets/19b6b859352a42c28710fb463af81697/nationell-strategi-diagnostik-covid-19-20081.pdf.
44. Västra Götalandsregionen. *Provtagningsindikationer - SARS-CoV2-IgG*. 2020 [cited 2020 16 Sept]; Available from: https://alfresco.vgregion.se/alfresco/service/vgr/storage/node/content/46404/Provtagningsanvisning%20-%20SARS-CoV2-IgG.%20Version%201.pdf?a=false&guest=true.
45. VGR Fokus. *Antikroppstester för allmänheten från vecka 40*. 2020 [cited 2020 23 Nov]; Available from: https://vgrfokus.se/2020/09/antikroppstester-for-allmanheten-fran-vecka-40/.
46. Folkhälsomyndigheten. *Genomförda tester för covid-19*. 2020 [cited 2020 11 Dec]; Available from: https://www.folkhalsomyndigheten.se/smittskydd-beredskap/utbrott/aktuella-utbrott/covid-19/statistik-och-analyser/genomforda-tester-for-covid-19/.
47. Augustine, R., et al., *Rapid Antibody-Based COVID-19 Mass Surveillance: Relevance, Challenges, and Prospects in a Pandemic and Post-Pandemic World.* Journal of clinical medicine, 2020. **9**(10).
48. Folkhälsomyndigheten. *Utvärdering av antikroppstester vid svenska laboratorier*. 2020 [cited 2020 24 Nov]; Available from: https://www.folkhalsomyndigheten.se/utokad-testning-for-covid-19/test-for-covid-19/pavisning-av-antikroppar/utvarderingar-av-antikroppstester-vid-svenska-laboratorier/#MainContent.
49. Västra Götalandsregionen. *Provtagning antikroppar covid-19 av vuxna, barn och hälso- och sjukvårdspersonal, version 2*. 2020 [cited 2020 23 Nov]; Available from: https://alfresco.vgregion.se/alfresco/service/vgr/storage/node/content/47737/Provtagni

ng%20antikroppar%20covid-19%20av%20vuxna,%20barn%20och%20h%C3%A4lso-%20och%20sjukv%C3%A5rdspersonal,%20version%201.pdf?a=false&guest=true.

50. Västra Götalandsregionen. *Regiontransporter*. 2020 [cited 2020 16 Sept]; Available from: https://regionservice.vgregion.se/RNS/prodnarservice/transport/budtransport/.
51. Sahlgrenska University Hospital. *SARS-CoV2-IgG, Covid-19-IgG*. 2020 [cited 2020 23 November]; Available from: https://sahlgrenska-klinkem-analyser.vgregion.se/KMIKAD110037.pdf.
52. Unilabs. *COVID-19 (SARS CoV-2 serologi)*. 2020 [cited 2020 16 Sept]; Available from: https://anvisningar.se/Anvisningar/Klinisk-mikrobiologi/C/COVID-19-SARS-CoV-2-serologi-/.
53. Unilabs. *Transporter*. 2019 [cited 2020 16 Sept]; Available from: https://anvisningar.se/Transporter/.
54. 1177 Vårdguiden. *Antikroppsteset för dig som har varit sjuk tidigare*. 2020 [cited 2020 23 Nov]; Available from: https://www.1177.se/Vastra-Gotaland/sjukdomar--besvar/lungor-och-luftvagar/inflammation-och-infektion-ilungor-och-luftror/om-covid-19--coronavirus/provtagning-for-covid-19-i-vgr/antikroppstest-vgr/.
55. Henriksen, L.O., et al., *Stability of 35 biochemical and immunological routine tests after 10 hours storage and transport of human whole blood at 21°C.* Scand J Clin Lab Invest, 2014. **74**(7): p. 603-10.
56. Hodgkinson, V.S., et al., *Preanalytical Stability of Antibodies to Pathogenic Antigens.* Cancer Epidemiol Biomarkers Prev, 2017. **26**(8): p. 1337-1344.
57. Dupuy, A.M., et al., *Stability of routine biochemical analytes in whole blood and plasma/serum: focus on potassium stability from lithium heparin.* Clin Chem Lab Med, 2018. **56**(3): p. 413-421.
58. Oddoze, C., E. Lombard, and H. Portugal, *Stability study of 81 analytes in human whole blood, in serum and in plasma.* Clin Biochem, 2012. **45**(6): p. 464-9.
59. Sahlgrenska University Hospital. *Centrifugering, förvaring*. 2020 [cited 2020 23 Nov]; Available from: https://sahlgrenska-klinkem-analyser.vgregion.se/KKAD106915.htm.

60. World Health Organization. *Coronavirus disease (COVID-19) Q&A*. 2020 [cited 2020 24 Nov]; Available from: https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19.
61. Folkhälsomyndigheten. *Om viruset och sjukdomen*. 2020 [cited 2020 24 Nov]; Available from: https://www.folkhalsomyndigheten.se/smittskydd-beredskap/utbrott/aktuella-utbrott/covid-19/om-sjukdomen-och-smittspridning/om-viruset-och-sjukdomen/.
62. Zhang, D.J., et al., *Effect of serum-clot contact time on clinical chemistry laboratory results.* Clinical Chemistry, 1998. **44**(6): p. 1325-1333.
63. Boyanton, B.L., Jr and K.E. Blick, *Stability Studies of Twenty-Four Analytes in Human Plasma and Serum.* Clinical Chemistry, 2002. **48**(12): p. 2242-2247.
64. Zhao, J., et al., *Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019.* Clinical Infectious Diseases, 2020.
65. Ma, H., et al., *Serum IgA, IgM, and IgG responses in COVID-19.* Cellular and Molecular Immunology, 2020. **17**(7): p. 773-775.
66. Biobank Sverige. *Nationella riktlinjer för preanalytisk hantering av vätskebaserade prov för forskning*. 2020 [cited 2020 03 Dec]; Available from: https://biobanksverige.se/wp-content/uploads/y2-nationella-riktlinjer-for-preanalytisk-hantering-av-vatskebaserade-prov-for-forskning.pdf.
67. Sahlgrenska University Hospital. *Chimerismstatus efter allogen stamcells-eller organtransplantation*. 2020 [cited 2020 03 Dec]; Available from: https://www.sahlgrenska.se/for-dig-som-ar/vardgivare/laboratoriemedicin/analyslista/t-celler/.
68. Sahlgrenska University Hospital. *Leukocyter Partikelkoncentraion*. 2020 [cited 2020 03 Dec]; Available from: https://www.sahlgrenska.se/for-dig-som-ar/vardgivare/laboratoriemedicin/analyslista/leukocyter/0243.html.
69. Sekine, T., et al., *Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19.* Cell (Cambridge), 2020. **183**(1): p. 158-168.e14.
70. West, R., et al., *COVID-19 Antibody Tests: A Valuable Public Health Tool with Limited Relevance to Individuals.* Trends in Microbiology, 2020.

71. Chen, X., et al., *Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19.* Signal Transduction and Targeted Therapy, 2020. **5**(1).
72. Shen, C., et al., *Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma.* JAMA, 2020. **323**(16): p. 1582-1589.
73. Duan, K., et al., *Effectiveness of convalescent plasma therapy in severe COVID-19 patients.* Proceedings of the National Academy of Sciences of the United States of America, 2020. **117**(17): p. 9490-9496.
74. Salazar, E., et al., *Treatment of Coronavirus Disease 2019 (COVID-19) Patients with Convalescent Plasma.* American Journal of Pathology, 2020. **190**(8): p. 1680-1690.
75. Fung, M., et al., *Treatment of Immunocompromised COVID-19 patients with Convalescent Plasma.* Transplant Infectious Disease, 2020.
76. Bloch, E.M., et al., *Deployment of convalescent plasma for the prevention and treatment of COVID-19.* The Journal of Clinical Investigation, 2020. **130**(6): p. 2757-2765.

Appendix

Appendix 1: The questionnaire filled out by participants to classify disease severity.

Blodgrupp [Blood type]:
Symtom start [Symptom debut]:
Varaktighet (dagar) [Symptom length (days)]:
Sjukhusvistelse [Hospital admittance]:
Behandling (t.ex. Alvedon, Ipren, annat) [Treatment (for example, Alvedon, Ipren, other)]:
Symtom (ja/nej) [Symptoms (yes/no)]: o Feber: [Fever] o Hosta: [Cough] o Dyspné: [Dyspnea] o Halsont: [Sore throat] o Rinnsnuva: [Runny nose] o Huvudvärk: [Headache] o Trötthet: [Tiredness] o Borttappning av lukt/smaksinne: [Loss of smell/taste] o Musklevärk/ledvärk: [Muscle/joint ache] o Diarée: [Diarrhea] o Buksmärta: [Stomach pain]
Andra kommentarer [Additional comments]: