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Coronavirus Pandemic

Stability of SARS-CoV-2 antibody in serum under various usage and storage conditions

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Abstract

Introduction: We investigated the effect of two preanalytical variables, temperature change and freezing-thawing of serum samples, on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG levels.

Methodology: Serum samples were collected from patients who had coronavirus disease 2019 (COVID-19) prior to vaccination. Six serum samples were included, two each with high positivity (HP), low positivity (LP), and a level of close-to-detection limit (CDL) for SARS-CoV-2 IgG. Each of these six samples was divided into three tubes and placed in refrigerators at 4-8 °C, -20 °C, and -70 °C; removed from the storage temperature once per day for 20 consecutive days; and assayed for SARS-CoV-2 IgG level.

Results: The coefficient of variation of all the remaining serum samples were within 95% except for CDL-1 serum at -70 °C, HP-2 serum at 4-8 °C, HP-2 serum at -20 °C, and HP-2 serum at -70 °C. The levels increased significantly when the temperature in the samples with CDL was reduced. The values in samples with LP at -20 °C and -70 °C were significantly higher than those at 4-8°C. In the case of samples with HP, the values of samples at -20 °C were higher than those in samples at 4-8 °C. There was no positive–negative change during any of the freeze-thaw cycles.

Conclusions: Antibody value in the samples at 4-8 °C remained stable throughout the 20 freeze-thaw cycles. The antibody value of the samples at -20 °C and -70 °C tended to elevate.

Key words: SARS-CoV-2 IgG; stability; serum; freeze-thaw.

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Introduction

The preanalytical period includes all procedures prior to laboratory analysis, including analysis request, patient preparation, sample collection, transportation, and temporary storage of the collected sample in laboratories that mainly work with blood, serum, and plasma samples. The quality and quantity of the collected sample or the data related to the sample are affected by errors that may occur during this period, and this is the most common cause of erroneous laboratory results. Preanalytical errors in routine health care necessitate extra effort to monitor and correct these errors, and delayed or erroneous analytical results may be harmful to the patient [1,2]. Improper storage of samples also contributes to preanalytical errors. Therefore, clinical laboratories should aim to minimize preanalytical errors to preserve sample composition and integrity, and prepare standard operating procedures and instructions in accordance with the published guidelines and studies [3,4].

Laboratory professionals should be aware of the factors affecting the analyte during thawing and reanalyzing stored samples. To obtain accurate test results, it is critical to ensure analyte stability in the stored samples and to be aware of the appropriate order of analysis, how to analyze the case and control samples to minimize variations in analyses, the effect of freeze-thaw cycles on the analyte, and which type of sample (plasma/serum) is suitable for storage. Research in this area provides guidance in the preparation of standard operating procedures [5]. Coronavirus disease 2019 (COVID-19) in a relatively new infection and when serum samples of COVID-19 patients need to be stored for more than five days, it is recommended to store them at -70 °C [6].

Precision is defined as “the distribution of measured results obtained under certain conditions” [7,8]. It measures the closeness or agreement between repeated independent measurements. After calibrating a laboratory experimental method for the first time, the

precision and trueness of the experiment are monitored using controls.

Precision is established by testing an assay's ability to deliver the same result in repeated measurements. The coefficient of variation (CoV%) value is used to test precision. The environmental conditions in the laboratory where the analysis is performed have a significant impact on the CoV% value. Environment-dependent variables include the temperature at which the reaction occurs, the source and quality of the reagents, and the repeatability of pipetting of the reagents [7-9]. The concept of "repeatability" under the same conditions, such as the same operator, reactive lots, tools, laboratory, time, and the concept of "reproducibility," as part of the particular analysis and on different days are important measures of precision [10].

The current study aims to investigate the effects of the two preanalytical variables, i.e., temperature change and freezing-thawing of serum samples, on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG test results. These two parameters were chosen because they may lead to erroneous test results even when working with the same technician, device, and SARS-CoV-2 IgG detection kit compatible with the device.

Methodology

A precision experiment was planned in accordance with the recommendations of the CLSI EP5-A2 document to examine the effects of the two preanalytical variables, i.e., temperature change and freezing–thawing of serum samples, on the antibody levels. The CLSI guidelines recommend that the experiment be carried out over at least 20 working days to ensure that each individual error component is adequately covered because there is high level of daily variation [7].

Figure 1. Coefficient of variation of antibody levels (y-axis) in close to detection limit (CDL) serum

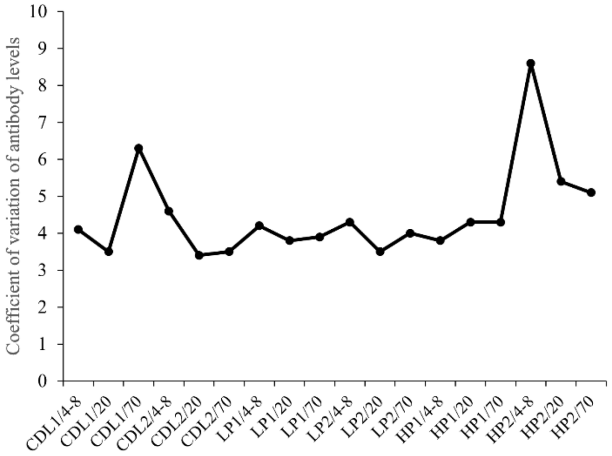


Table 1. Basal SARS-CoV-2 IgG antibody levels of the selected serum.

Serum	Antibody levels
CDL-1	2.96
CDL-2	4.77
LP-1	29.26
LP-2	45.64
HP-1	4199
HP-2	10386

CDL: close-to-detection limit; LP: low positivity; HP: high positivity; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 IgG level tests of the patients were routinely requested in the Microbiology Laboratory of our hospital. The tests were carried out using the "SARS- CoV-2 IgG" kit on the VIDAS device (bioMerieux, Marcy-l' Étoile, France) to measure antibodies against the spike (S) protein of the virus. Serum samples were collected from patients who had COVID-19 before the initiation of vaccination in Turkey, and who tested positive in reverse transcriptase polymerase chain reaction (RT-PCR) tests for COVID-19. The samples that tested positive for SARS-CoV-2 IgG were stored at -70 °C until the study began.

To assess the effect of temperature change on the test result, two serum samples with high positivity (HP), two with low positivity (LP), and two with a level of close-to-detection limit (CDL) for SARS-CoV-2 IgG were selected from among the stored serum samples and used in the study. The baseline level of SARS-CoV-2 antibodies in the samples were measured at the beginning of the study. The serum samples were brought to room temperature and analyzed on the immunoassay analyzer (Cobas e 411, Roche Diagnostics, Mannheim, Germany) using the electro-chemiluminescence immunoassay kit (Elecsys Anti-SARS-CoV-2 S, Roche, Mannheim, Germany) that is designed for quantitative assessment of antibodies against the receptor-binding domain of the viral S protein. The device automatically calculates the positive values quantitatively. Test results of < 0.80 U/mL were considered negative and those of > 0.80 U/mL were considered positive.

All the serum samples were then divided into portions and stored in refrigerators at 4-8 °C, -20 °C, and -70 °C; removed from the storage temperature 1 time per day for 20 consecutive days; and assayed for SARS-CoV-2 IgG levels after bringing them to room temperature. Since there were not enough kits available, the serum samples with CDL levels were measured for only 16 consecutive days.

Table 2. Analysis of SARS-CoV-2 IgG antibody levels according to temperature change.

Levels of the serum	Min-Max	Median	Ort. ± ss	p*		p‡	
Close to detection limit (CDL)							
4–8 ºC	2.60–5.11	3.63	3.65 ± 0.91				
-20 ºC	2.69–4.87	3.66	3.75 ± 0.87	**0.000**	w		
-70 ºC	2.75–5.01	3.98	3.83 ± 0.89	**0.000**	w	**0.000**	w
With low positivity (LP)							
4–8 ºC	26.44–47.70	35.82	36.04 ± 7.90				
-20 ºC	27.73–47.40	36.79	37.06 ± 7.80	**0.000**	w		
-70º	27.53–48.81	36.96	37.39 ± 8.44	**0.000**	w	0.154	w
With high positivity (HP)							
4–8 ºC	3959–10828	5618	6942 ± 2840				
-20 ºC	3978–10803	6656	7101 ± 2845	**0.010**	w		
-70 ºC	3985–	6808	7035 ± 2822	0.888	w	0.107	w

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2. Significant *p* values are marked in bold letters.

The control and calibration for this study were carried out in accordance with the manufacturer's recommendations. SARS-CoV-2-S IgG values were measured over a period of 20 days and results were interpreted after determining their standard deviation and CoV% values.

Statistical analysis

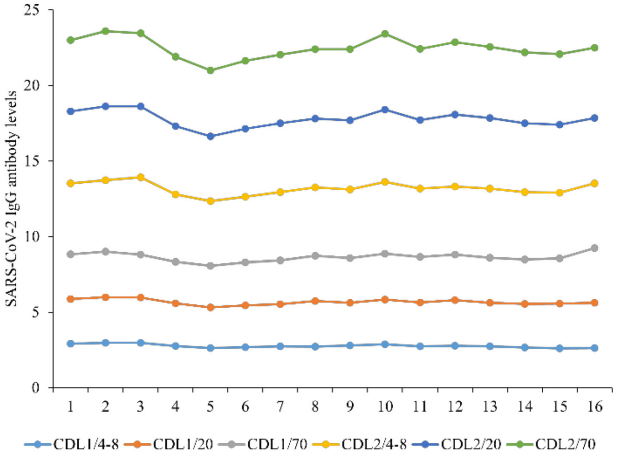
Mean, standard deviation, and median lowest and highest values were used in the descriptive statistics of the data. The distribution of the variables was assessed by the Kolmogorov–Smirnov test. The Wilcoxon test was used to analyze dependent quantitative data. The SPSS 28.0 program (IBM Corp, Armonk, NY, U.S.A.) was used to conduct the statistical analyses.

Results

The baseline antibody levels of the selected serum samples with HP, LP, and a level of CDL are presented in Table 1.

The CDL serum samples were analyzed for 16 consecutive days, and the HP and LP serum samples were analyzed for 20 consecutive days following daily freeze-thaw process. Accordingly, the min-max, standard deviation, and median values were calculated and are presented in Table 2. In terms of the CoV% value of the antibody measurements, the measured value of antibody level was 6.3 for the CDL-1 serum stored at -70 °C, 8.6 for the HP-2 serum stored at 4-8 °C, 5.4 for the HP-2 serum stored at -20 °C, and 5.1 for the HP-2 serum stored at -70 °C. For the antibody measurements of the remaining serum samples, the CoV% levels at all temperatures were within 5% (Figure 1).

Figure 2. SARS-CoV-2 IgG antibody levels (y-axis) in close to detection limit (CDL) serum.



SARS-CoV-2 IgG antibody levels (y-axis) in close to detection limit (CDL) serum.

SARS-CoV-2 IgG antibody levels of the serum with CDL, LP, HP are shown in Figures 2-4. In terms of the effect of temperature change on the analyte, there was a significant increase in the analyte measurement due to the reduction in the temperature ($p < 0.05$) in the serum with a level of CDL. In the serum samples with LP, the values of the samples stored at -20 °C and -70 °C were significantly higher than those at 4-8 °C ($p < 0.05$), but the values measured at -70 °C were not significantly higher compared to those measured at -20 °C ($p > 0.05$). In the case of serum samples with HP, the values measured in the samples stored at -20 °C were higher than those measured at 4-8 °C ($p < 0.05$); the values of the samples stored at -70 °C did not have any significant elevation ($p > 0.05$); and the values measured in the samples stored at -70 °C did not differ from those at -20 °C ($p > 0.05$) (Table 2). There was no positive-negative change during any of the freeze-thaw cycles.

Discussion

Our study presents a precision assessment and statistical analysis on variation in order to investigate the effects of two preanalytical factors – temperature change and freeze-thaw cycles – on serum antibody levels.

Figure 3. SARS-CoV-2 IgG antibody levels (y-axis) in low positivity (LP) serum.

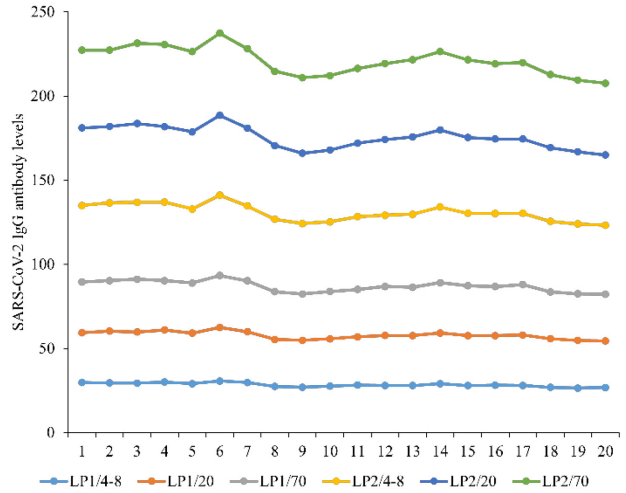
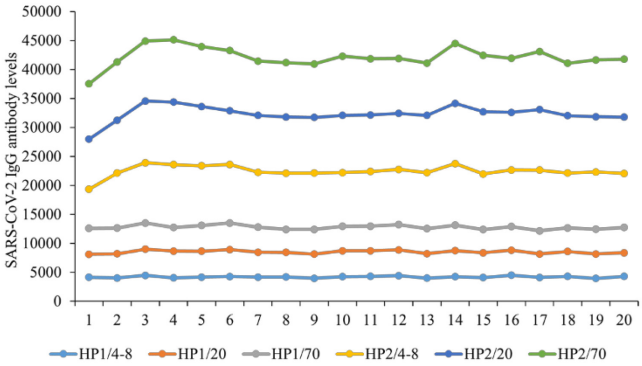


Figure 4. SARS-CoV-2 IgG antibody levels (y-axis) in high positivity (HP) serum.



It is recommended to store the samples by portioning them in appropriate amounts to eliminate and control the changes that may occur during storage and repetitive freeze–thaw cycles. Storage media (serum and plasma), storage time, and the number of freeze-thaw cycles are among the main control parameters [9]. Given the antigen-specific affinity of antibodies, changes in the chemical structure of the antibody in stored serum/plasma are critical. Structural changes or degradation in antibodies due to storage conditions may lead to reduced antibody activity, thereby producing false negative results [4].

A study by Freiburghaus *et al.* investigated the effects of freeze-thaw cycles on some parameters (sodium, potassium, calcium, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), lipase, uric acid, albumin, C-reactive protein, and total protein), and concluded that calcium, ALT, and total protein were sensitive to all the three freeze-thaw cycles performed in the study [11]. They observed that the remaining parameters changed significantly after at least one cycle, with each cycle of testing leading to changes in analyte concentration or activity in at least three of the ten parameters compared to the unfrozen form. They suggested that the effect of freeze–thaw cycles, one of the preanalytical factors affecting the quality of the stored sample, is not completely understood and needs to be further investigated.

Hepatitis C virus (HCV)-RNA levels and serum/plasma stability at room temperature were reported to significantly decrease at 24, 48, and 72 hours in the initial studies [12,13]; but they remained unchanged in subsequent studies [14-16]. While some studies have reported that the number of freeze-thaw cycles has no effect on HCV-RNA levels [16-18], others have reported that the levels decrease [19]. Researchers studying antibodies concluded that antibodies for measles, rubella, and mumps remained stable throughout 10 freeze-thaw cycles [20]. However, anti-HIV antibodies were reduced in reactivity when stored at 25°C for a long time; there was a significant loss of reactivity when stored at 37 °C for 21 days, and they remained positive even after 57 days despite the loss of reactivity [21]. In other studies, IgG and IgM-type antibodies against syphilis did not lead to a significant loss of sensitivity in serum after 10 freeze-thaw cycles [22]. Researchers have also reported that IgA, IgG, and IgM antibody activity levels measured for *Mycoplasma pneumoniae*, *Yersinia enterocolitica*, and *Salmonella* antigens were stable even after 30 freeze-thaw cycles [23].

Two previous studies have examined the change in SARS-CoV-2 antibodies under various temperatures. Shurrab *et al.* investigated the effect of multiple freeze–thaw cycles on SARS-CoV-2 IgG detection in serum using qualitative enzyme linked immunosorbent assay (ELISA) targeting nucleocapsid (N) antibodies and showed that positivity changed to an indefinite value in 0.8% of the positive measurements and negativity changed to an indefinite value in 20% of the negative measurements, indicating that there was no change from positive to negative or negative to positive throughout the cycles [24]. Kanji *et al*. examined the change in SARS-CoV-2 antibodies under various temperatures using qualitative tests for SARS-CoV-2 IgG and demonstrated that the detected antibodies remained robust and stable at various storage temperatures for up to 12 freeze-thaw cycles [25].

Unlike the two studies above, we analyzed the SARS-CoV-2 S IgG levels using the quantitative ELISA kit. In our precision assessment, it was found that most of the serum samples remained within the expected CoV% values. The serum samples with a CoV

of > 5% were CDL-1/70 °C (6.3), HP-2/4-8 °C (8.6), HP-2/20 °C (5.4), and HP-2/70 °C (5.1). When the measurements were examined using a day-based approach, the first day measurements of HP-2/4-8 °C (8.6), HP-2/20 °C (5.4), and HP-2/70 °C (5.1) serum samples were lower than the baseline value. Furthermore, when the first-day values were excluded, the CoV% values of all except HP-2/70 °C improved. HP-2/4-8 °C decreased from 8.6 to 4.8 and HP-2/-20 °C decreased from 5.4 to 4.7, while HP-2/-70 remained unchanged.

The CDL-1/70 °C serum values were examined using a day-based approach. However, contrary to measurements in other serum samples, there was no change on the first day. In this case, it was suspected that the device had a random problem on that day.

In this study, we investigated the effect of temperature change on the analyte. The level of analyte in the CDL serum was significantly elevated after the temperature was reduced. This finding suggests that false positivity may occur in serum samples stored at -20 °C or -70 °C, when the antibody level is lower than the amount of serum used or falls within an indefinite range.

It is recommended that future study designs include evaluating the samples in duplicate twice a day over twenty working days. This type of study design will allow calculation of within-run, between-run, and between-day variances, which can then be combined to determine the total variance of the assay [6]. In our study, financial support for the supply of the antibody-detecting kit was limited; therefore, the test was run once a day for 20 days. This was a limitation of our study.

Conclusions

Our study demonstrated that the antibody level in the serum samples stored at 4-8°C remained stable throughout 20 freeze-thaw cycles, and the antibody levels of the serum samples stored at -20 °C and -70 °C tended to increase compared to those stored at 4-8 °C. Therefore, serum antibody levels that are lower than the CDL may be erroneously interpreted as false positives. The upward trend in low and high positive values in the presence of temperature reduction was not clinically significant in this study because these samples were already positive. However, since the protective limit value of the antibody is unknown, it would be appropriate to conduct further studies on stability in the future to reveal the clinical significance of these minor changes.

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Ethics approval

This study was approved by the Bulent Ecevit University Clinical Research Ethics Committee (Decision No: 2021/04).

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