Heliyon 10 (2024) e34449

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Blood collection tube and anticoagulant influence on SARS-CoV-2 antibody and avidity levels

Nicholas C. Castro, Jimmie Bullock, Katarzyna Haynesworth, Sarah Loftus, Jordan Metz, Hayley North, Troy J. Kemp, Ligia A. Pinto *

Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD, 21702, USA

A B S T R A C T

SARS-CoV-2 serology plays a crucial role in assessing COVID-19 vaccine immunogenicity and antibody responses to SARS-CoV-2 infection. Tube type and anticoagulant may influence serology results. Thus, understanding the influence of these variables in test results is key.

We evaluated the influence of serum collection tube type and anticoagulant on anti-SARS-CoV-2 spike antibody levels detected by enzyme-linked immunosorbent assays (ELISAs) and Luminex multiplex assays (11-plex) in serum and plasma samples. Anti-spike IgG avidity was also evaluated in both sample types.

No significant differences were found between serology assay results using different blood (serum) collection tube types. However, significantly lower antibody concentrations ($p < 0.05$) were observed in tubes with the anticoagulants sodium citrate and acid citrate dextrose (ACD) in the ELISA and Multiplex assays ($n = 29$), compared to expected concentrations. These differences mostly disappeared after adjusting for the dilution factor caused by the anticoagulant volume, indicating that anticoagulant does not significantly impact the assay results, while anticoagulant volume does.

There was a significant difference ($p < 0.05$) in IgG avidity (M) of plasma samples ($p < 0.05$) compared to serum, but anticoagulant type had no effect.

Overall, these findings indicate that the choice of collection tube may introduce subtle variations in assay results if the volumes of anticoagulants are not taken into consideration. Additionally, differences between serum and anticoagulant-treated plasma matrices were observed in avidity ELISAs, indicating that these samples are not interchangeable for these assays; a finding that requires further investigation.

1. Introduction

The emergence of the SARS-CoV-2 virus (COVID-19) in 2019 posed a global threat to public health [1]. SARS-CoV-2 infection can manifest as asymptomatic or cause severe respiratory distress, potentially leading to hospitalization and mortality [1]. Individuals with comorbidities such as hypertension, diabetes, cardiovascular disease, chronic pulmonary disease, chronic kidney disease, and/or malignancy have a higher risk of hospitalization or death upon infection [1,2]. To control the highly transmissible SARS-CoV-2, intra-muscular ribonucleic acid (RNA) based vaccines (BNT162b2 and mRNA-1273) targeting the Spike protein of SARS-CoV-2 were developed and made available at an unprecedented pace, later demonstrating high levels of efficacy against hospitalization and death [3,4]. A correlate of protection against infection conferred by these vaccines, however, is still being investigated.

SARS-CoV-2 serology can be used to evaluate the immunogenicity of participants following COVID-19 vaccinations or infection. In general, blood from clinical and research studies have been collected using sterile collection tubes (STs), including glass (GTs), plastic (PTs), or gel separation collection tubes (GSTs) which separate the serum through gel filtration. These different types of tubes have

* Corresponding author. 1050 Boyles Street Building 469, Room 110, Frederick, MD, 21702, USA.
E-mail address: pintol@mail.nih.gov (L.A. Pinto).

https://doi.org/10.1016/j.heliyon.2024.e34449
Received 17 January 2024; Received in revised form 9 July 2024; Accepted 9 July 2024
Available online 10 July 2024

subtle variations that may influence serology assay results [5]. An additional variable is the presence or absence of anticoagulants to stabilize the sample and prevent coagulation [6]. Different anticoagulants have been used in various studies, including acid citrate dextrose (ACD), sodium heparin, sodium citrate, or ethylenediaminetetra acetic acid (EDTA) (Table 1) [7,8]. To our knowledge, the potential impact of collection tube type for serum collection and anticoagulant for plasma on SARS-CoV-2 serology results have not been formally evaluated. The goals of this study are to investigate the influence of collection tube type and anticoagulant on serology assay (enzyme-linked immunosorbent assay [ELISA], avidity enzyme-linked immunosorbent assay [chaotropic ELISA], and a Luminex-based multiplex assay [11-Plex]) results.

2. Results

2.1. Effect of serum collection tube on serology assays results

No significant differences were observed for anti-SARS-CoV-2 Spike IgG ELISA or Luminex anti-SARS-CoV-2 Spike IgG (Luminex CoV2_S) concentrations (binding antibody units [BAU]/mL) in serum collected in glass, plastic, or gel separator tubes (Friedman Rank Sum: ELISA, $p = 0.2285$; and Luminex CoV2_S, $p = 0.0511$; Fig. 1A and B). In addition, there were similar results for all antibodies against the other 10 targets (CoV2_N, R_RBD, R_RBD_UK, R_RBD_E484K, M_RBD, M_RBD_UK, M_RBD_SA, M_RBD_E484K, CoV1_S, MERS_S, summarized in Table S1) analyzed via the Luminex platform (Fig. S1). Furthermore, no significant differences were detected in the mean anti-SARS-CoV-2 Spike IgG avidity indices (Molarity [M]) across different serum collection tube types ($p = 0.7165$; Fig. 1C).

To further evaluate if any differences presented in serology results, anti-SARS-CoV-2 Spike IgG ELISA, Luminex CoV2_S, and anti-SARS-CoV-2 Spike IgG avidity levels were directly compared between assays conducted in serum samples collected in gel versus glass tubes, or plastic versus glass tubes. Results were highly correlated between gel and glass across all 3 assays (Spearman: ELISA, $\rho = 0.997$; Luminex CoV2_S, $\rho = 0.989$; and Avidity, $\rho = 0.951$, all correlations were $p < 0.0001$) (Fig. 1D–F). Results were also highly correlated between plastic and glass for the 3 assays (Spearman: ELISA, $\rho = 0.992$; Luminex CoV2_S, $\rho = 0.995$; and Avidity, $\rho = 0.928$, all correlations were $p < 0.0001$) (Fig. 1D–F).

2.2. Effect of anticoagulant on serology assay results

Anticoagulants significantly affected the anti-SARS-CoV-2 Spike IgG ELISA antibody concentrations, specifically when serum samples collected in glass tubes were compared with plasma samples treated with ACD or sodium citrate ($p = 0.0016$ and 0.0022, respectively; Fig. 2A). However, these differences were resolved when the ACD or sodium citrate anti-coagulant volumes were adjusted for dilutional effects (Friedman rank sum test, $p = 0.2937$; Fig. 2A).

Similarly, significant differences in mean Luminex CoV2_S antibody concentrations (BAU/mL) were observed between serum samples collected in glass tubes and plasma samples treated with ACD ($p = 0.0024$) and sodium citrate ($p = 0.0017$; Fig. 2B). The difference remained even after accounting for the added sodium citrate volume for the Luminex CoV2_S assay when compared to serum glass tubes ($p = 0.0280$; Fig. 2B). Other significant differences were also observed for the other 10 proteins tested on the Luminex platform (Table S1, Fig. S2).

For the avidity assay, significant differences were observed between serum samples collected in glass containers compared to plasma samples treated with ACD ($p = 0.0041$), EDTA ($p = 0.0014$), or sodium citrate ($p = 0.0140$) (Fig. 2C). However, no significant difference ($p > 0.05$) was observed among the plasma samples treated with varying anticoagulants.

Spearman correlation coefficients were calculated for all 3 assays (CoV2_S only for Luminex) for plasma ACD (adjusted), plasma

Table 1
Description of study collection tubes.

Study	Sample Size (n)	Defining Characteristic	Plasma or Serum Sample	Glass or Plastic	Blood Draw Volume (mL)	Additive Concentration	Additive Volume (mL)	Percentage of Additive (v/v)
Container Material Comparison (Serum)	21	Gel	Serum	Plastic	8.5	Clot activator/polymer gel	0	
		Plastic	Serum	Plastic	10	Clot activator. Silicone coated	0	
		Glass	Serum	Glass	10	Silicone coated	0	
Anticoagulant Comparison (Plasma and Serum)	29	EDTA	Plasma	Plastic	10	K2EDTA 18 mg	0	
		Sodium Citrate	Plasma	Plastic	2.7	Buffered sodium citrate (0.109 M, 3.2 %)	0.3	10 %
		ACD	Plasma	Glass	8.5	Acid citric dextrose (ACD) solution A consists of trisodium citrate, 22.0 g/L, citric acid, 8.0 g/L, and dextrose, 24.5 g/L	1.5	15 %
		Sodium Heparin	Plasma	Plastic	10	Sodium Heparin 158 USP units	0	
		Glass	Serum	Glass	10	Silicone coated	0	

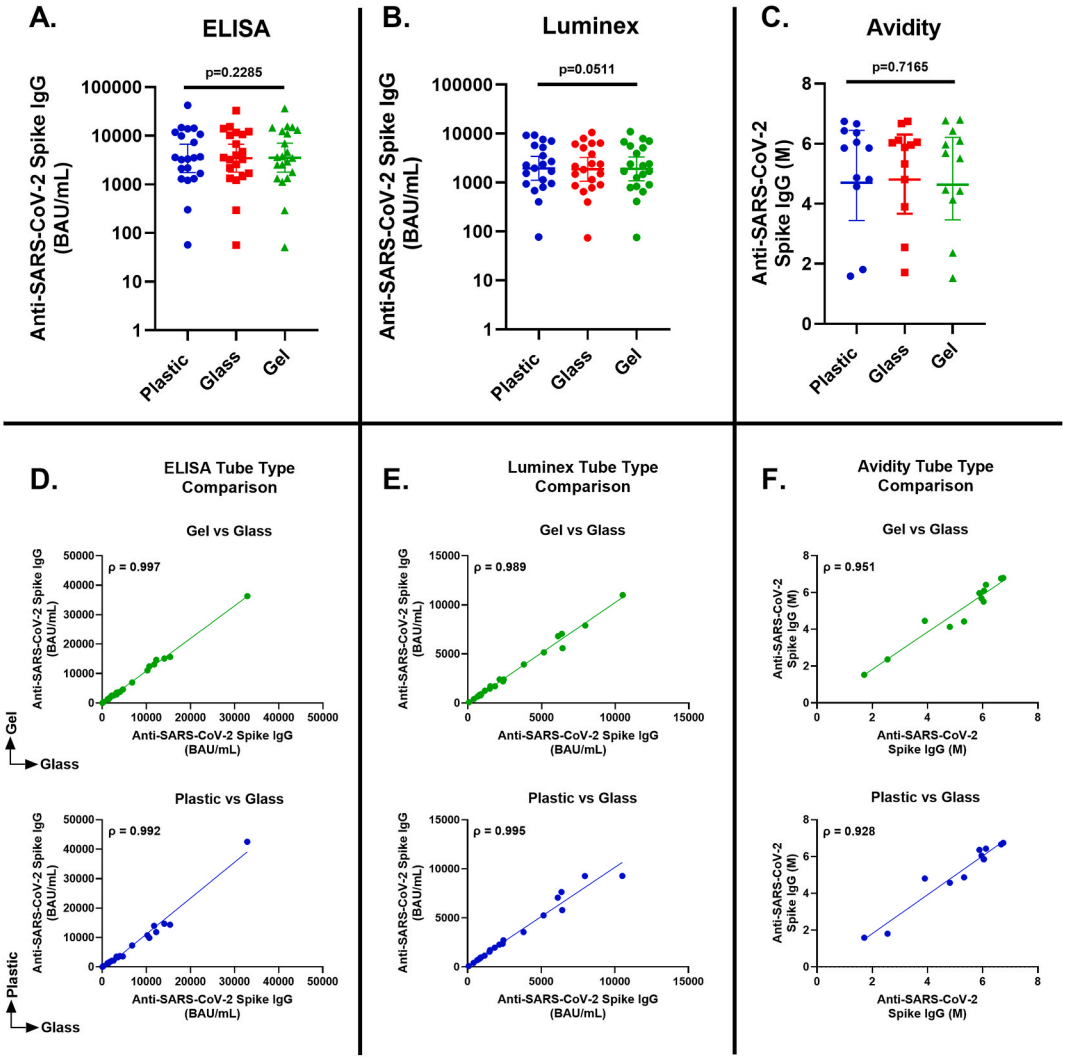
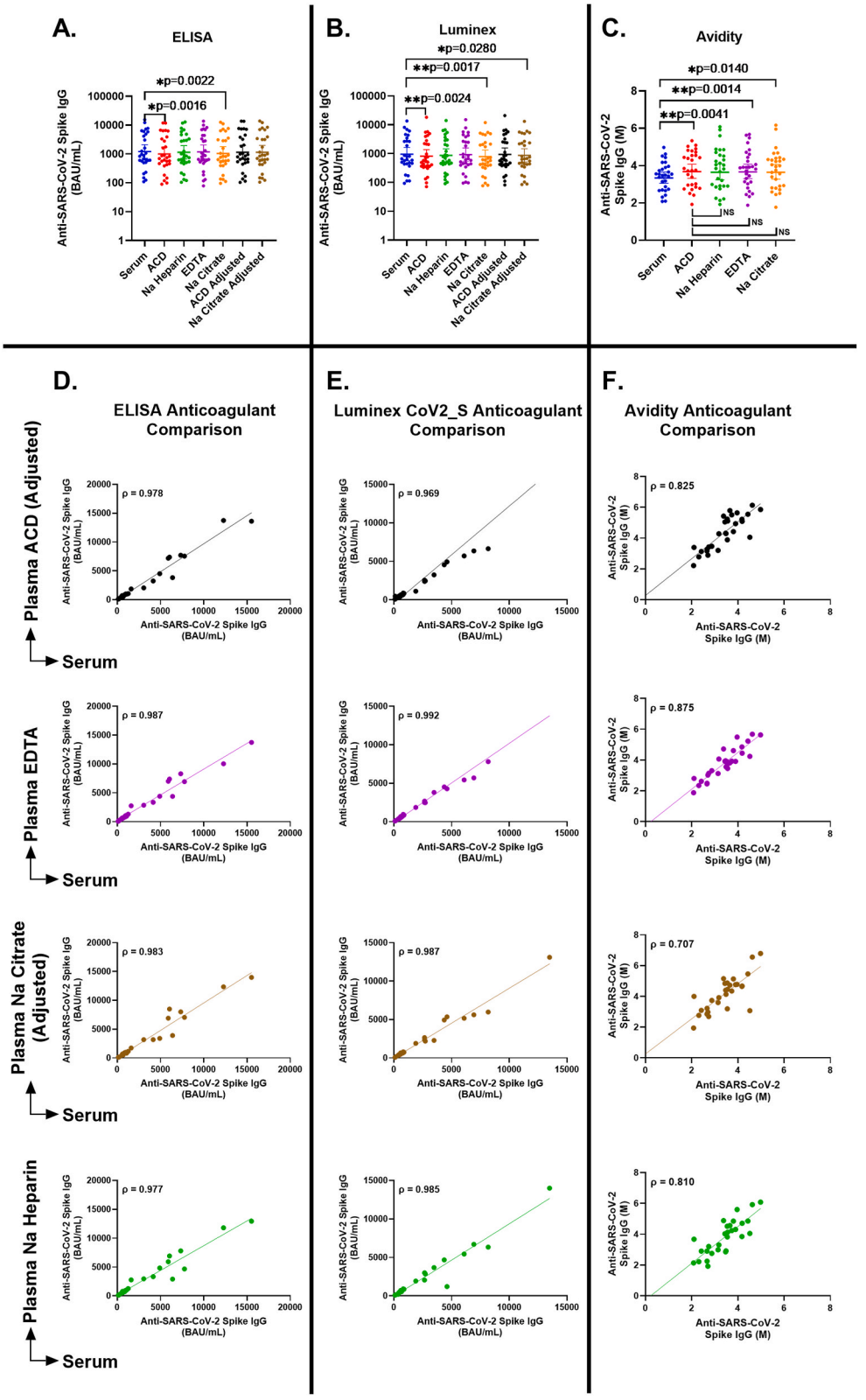


Fig. 1. Effects of Serum Tube Collection Type on Serology Assay Results. The scatter dot plots (A–C) include all sample results, while the bold horizontal bar represents the geometric mean (GM) and the error bars represent the 95 % confidence interval (CI). A) Anti-SARS-CoV-2 Spike IgG ELISA assay (Friedman Rank Sum p-value across the three serum collection tubes, p = 0.2285) and B) Luminex Multiplex assay analyzing anti-SARS-CoV-2 Spike IgG concentrations of donors collected in various serum sample collection tubes containing serum samples (Friedman Rank Sum p-value across the three serum collection tubes, p = 0.0511). C) Avidity (Chaotropic ELISA) assay analyzing anti-SARS-CoV-2 Spike IgG avidity indices (M) of donors collected in various serum sample collection tubes (Friedman Rank Sum p-value across the three serum collection tubes, p = 0.7165). D-F) Non-parametric Spearman correlation graphs illustrating a strong correlation between the samples, and the p-value for all graphs (D–F) were significant ($p < 0.0001$). D) Anti-SARS-CoV-2 Spike IgG ELISA antibody responses for Gel versus Glass (Spearman correlation coefficient (ρ), $\rho = 0.997$) and Plastic versus Glass ($\rho = 0.992$). E) Luminex anti-SARS-CoV-2 Spike IgG antibody responses for Gel versus Glass ($\rho = 0.989$) and Plastic versus Glass ($\rho = 0.995$). F) Anti-SARS-CoV-2 Spike IgG avidity indices (M) for Gel versus Glass ($\rho = 0.951$) and Plastic versus Glass ($\rho = 0.928$).

EDTA, plasma Sodium Citrate (adjusted), and plasma Sodium Heparin compared to serum (no anticoagulant). Spearman correlations for anti-SARS-CoV-2 Spike IgG ELISA results were as follows: serum glass versus plasma ACD (adjusted), $\rho = 0.978$, $p < 0.0001$; serum glass versus plasma EDTA, $\rho = 0.987$, $p < 0.0001$; serum glass versus plasma Sodium Citrate (adjusted), $\rho = 0.983$, $p < 0.0001$; serum glass versus plasma Sodium Heparin, $\rho = 0.977$, $p < 0.0001$ (Fig. 2D). All Spearman correlations were significant ($p < 0.0001$) for Luminex CoV2_S assay, and the correlation coefficients were as follows: serum glass versus plasma ACD (adjusted), $\rho = 0.969$; serum glass versus plasma EDTA, $\rho = 0.992$; serum glass versus plasma Sodium Citrate (adjusted), $\rho = 0.987$; serum glass versus plasma Sodium Heparin, $\rho = 0.985$ (Fig. 2E). For anti-SARS-CoV-2 Spike IgG avidity assay, all Spearman correlation combinations were significant ($p < 0.0001$), and the correlation coefficients were as follows: serum glass versus plasma ACD (adjusted), $\rho = 0.825$; serum glass versus plasma EDTA, $\rho = 0.875$; serum glass versus plasma Sodium Citrate (adjusted), $\rho = 0.707$; serum glass versus plasma Sodium Heparin, $\rho = 0.810$ (Fig. 2F).



(caption on next page)

Fig. 2. Effects of Anticoagulant on Serology Assay Results. The scatter dot plots (A–C) include all sample results, while the bold horizontal bar represents the geometric mean (GM) and the error bars represent the 95 % confidence interval (CI). A) GM and 95 % CI for anti-SARS-CoV-2 Spike IgG ELISA assay indicating a significant difference between serum (glass tube) and plasma samples containing ACD ($p = 0.0016$) or sodium (Na) citrate ($p = 0.0022$) prior to dilution correction. B) GM and 95 % CI for Luminex COV2_S assay analyzing anti-SARS-CoV-2 Spike IgG concentrations indicating a significant difference between serum (glass tube) and plasma samples containing ACD ($p = 0.0024$) or Na citrate ($p = 0.0017$) prior to dilution correction. Even after dilution correction, Na citrate was still significantly different from serum (glass tube) ($p = 0.0280$) C) GM and 95 % CI for Avidity (Chaotropic ELISA) assay analyzing anti-SARS-CoV-2 Spike IgG avidity indices (M) indicating a significant difference between all plasma samples as compared to serum (glass tube) samples (Serum:Plasma ACD, $p = 0.0041$; Serum:Plasma EDTA, $p = 0.0014$; Serum:Plasma Na citrate, $p = 0.0140$). No significant differences were observed between plasma samples containing various anticoagulants within the Avidity assay. D-F) Anticoagulant type versus serum (glass tube) sample comparison graphs for each assay (D, Anti-SARS-CoV-2 Spike IgG ELISA; E, Luminex COV2_S assay; F, anti-SARS-CoV-2 Spike IgG avidity assay). The Spearman correlation coefficients illustrate a strong correlation between the sample comparisons as all were significant ($p < 0.0001$). NS- not significant.

3. Discussion

In this study, the influence of various serum collection tube types and anticoagulants on serology assay results was investigated. Results showed different serum collection tubes did not affect anti-SARS-CoV-2 spike IgG ELISA, Luminex CoV2_S, or anti-SARS-CoV-2 spike IgG avidity assay results for serum samples. However, there was a significant difference between serum samples and plasma samples collected in tubes with the anticoagulants ACD or sodium citrate ($p < 0.05$). Further investigation of the vendor information regarding the tubes revealed that both the ACD and sodium citrate tubes contained a volume of their respective anticoagulants, instead of a coating or powder as in the EDTA and sodium heparin tubes (Table 1). The presence of this extra volume in the tube diluted the sample within the tube by approximately 15 % and 10 % for ACD and sodium citrate, respectively, prior to dilution of the samples for the assays. A re-analysis was conducted after adjusting the mean antibody concentrations (BAU/mL) by a factor of 1.15 and 1.10 for the ACD and sodium citrate volumes, respectively, for each of the samples. This necessary value adjustment eliminated the significant differences between the serum glass tubes and the ACD or the sodium citrate tubes ($p > 0.05$) for anti-SARS-CoV-2 spike IgG ELISA assays (Fig. 2A), but not for sodium citrate in the Luminex CoV2_S assay ($p = 0.0280$, Fig. 2B). Similar differences were also apparent for other markers targeted in the 11-plex Luminex assay (Fig. S2). These enduring differences in the Luminex assay warrant further investigation and may be due to experimental variation. In most cases, however, these results suggest that if dilutions are carefully considered, no significant differences should be observed between any of the plasma samples as compared to the serum samples.

These findings provide evidence that the sample volume introduced by the anticoagulant in the plasma tubes can have a substantial impact on assay results. Thus, careful consideration should be given to the choice of anticoagulant and its associated volume when performing serology assays. In this study, antibody concentration adjustment was done after seeing the data. In the future, such volumes should be incorporated in the initial results calculation to account for the anticoagulant volume present in the tubes. However, this additional step can be overlooked when sample collection and aliquoting occur at different facilities and indicate a need for proper communication between testing laboratories and blood collection sites.

In the case of the effects of anticoagulants on anti-SARS-CoV-2 spike IgG Avidity assay results, our study demonstrated that plasma samples treated with ACD, EDTA, or sodium citrate exhibited significant differences ($p < 0.05$) compared to the serum sample, which was not treated with an anticoagulant (Fig. 2C). These results indicate that serum and plasma are not interchangeable for use in the anti-SARS-CoV-2 spike IgG avidity assay, as the anticoagulant is likely to influence results of this avidity assay.

These results are not unexpected. Previous studies have investigated the role of tube type or anticoagulants on assay results in blood samples, with various results [5,6,8–10]. While this study demonstrated no effects of tube type on antibody levels or avidity, tube type has been shown to have an effect on analytes such as oxidative stress, hormone, and tumor markers [5,9,10]. In 2005, Smets et al. published a study demonstrating that assays measured significantly different ($p < 0.05$) pregnancy-associated plasma protein-Ak free triiodothyronine, and prostate-specific antigen levels depending on if the sample was collected in a plastic or a glass tube [10]. Bowen et al. (2014) reported changes in measured total thyroxine levels measured in Vacuette™ tubes ($p = 0.0037$) or BD Vacutainer serum separator tubes ($p = 0.0001$) compared to glass tubes [9]. In 2022, Bastin et al. found that nitric oxide, ferric reducing ability of plasma, and total thiol levels were significantly lower in samples collected in gel separator tubes and/or plain plastic tubes compared to samples collected in glass tubes [5]. In addition, anticoagulant presence and type has been demonstrated to have an effect on bacterial colony forming unit (CFU) formation and intracellular growth or blood cell metabolic profile [6,8]. These results indicate that tube type or additives (such as anticoagulants) can have significant effects on measurand, as is seen for avidity in this study.

Our results demonstrated some differences in antibody binding and avidity levels in serum compared to plasma samples. However, antibody avidity did not appear to differ between plasma samples treated with different anticoagulants. These findings indicate that anticoagulant presence, but not type, can affect antibody binding strength measures, or that the difference lies in antibodies in plasma matrices versus serum. Plasma is different from serum in that it has lower amounts of competing proteins, higher viscosity, fibronectin/other clotting factors, and lower potassium, lactate dehydrogenase, coagulation factor activation peptides, thromboglobulins, and other platelet components [11]. Different types of anticoagulants such as Potassium EDTA or heparin have also been demonstrated to interact with clinical chemistry assay reagents, affecting results [11]. Further research is necessary to determine whether the anticoagulants used in this study can interact with the antigen assay components or directly impact antibody avidity, or if the change may be due to interactions between the antibodies and the different matrices.

There is currently no clear guidance on tube type or anticoagulants for the collection of samples from patients infected or vaccinated for SARS-CoV-2. These results indicate that additional guidelines may be necessary to further standardize SARS-CoV-2 serology.

In the meantime, it will be important to use the same protocol for collection, including the type of tube within a study and to carefully record the type of tube used when performing serology assays, so results may be appropriately compared against other studies.

4. Conclusion

In conclusion, our study investigated the influence of serum collection tube type and anticoagulants on serum and plasma samples, respectively, on various serological assays. We found that the type of blood collection tube (glass, plastic, or gel separator tube) to obtain serum had no significant effect on assay results for anti-SARS-CoV-2 spike IgG ELISA, anti-SARS-CoV-2 spike IgG Avidity, and anti-SARS-CoV-2 spike IgG Luminex assays.

Significant differences on the anti-SARS-CoV-2 Spike IgG ELISA and Luminex were initially observed when comparing plasma samples with different anticoagulants to serum samples. However, following adjustments for anticoagulant volumes and dilution factors, most of the significant differences were eliminated.

The lack of significance observed for ELISA and most of the Luminex comparisons indicate that the choice of anticoagulant does not significantly impact binding assays when taking into consideration the dilution factors introduced by the volumes of anticoagulants in the tubes. However, significant effects were seen on avidity, where anticoagulant presence (but not type) exhibited significant differences in antibody avidity compared to the serum sample. Further investigations are needed to understand the underlying reasons for the observed differences between serum and plasma matrixes in avidity assays.

Due to the limitations of the Luminex data, including the small sample size, further studies using larger sample populations are warranted to conclusively determine the potential influence of anticoagulants on serology assays utilizing the Luminex platform.

In summary, our findings highlight the importance of carefully evaluating and considering the potential influence of serum collection tube type and anticoagulant for plasma collection, as they can introduce variations in assay results including additive volumes already present within the collection tubes. Our findings also indicate that plasma and serum samples are not interchangeable in avidity assays, and one matrix should be utilized for the duration of the study. Understanding these effects is crucial prior to conducting blood collections and accurate and reliable serology testing.

5. Materials and methods

5.1. Samples

Human serum or plasma samples (n = 21 for collection tube type; n = 29 for anticoagulant comparison) were collected by Occupational Health Services (OHS), National Cancer Institute (NCI) Frederick, Frederick MD, under the Research Donor Protocol (NIH OH99CN046), and all donors consented to blood collections. The donors' samples were deidentified at collection, so the processing laboratory and testing laboratory were blinded to the identification of the donors. The collections occurred between April 6, 2021 and September 13, 2022. The processing laboratory isolated serum and plasma from whole blood in preparation for testing. Blood donors in this study were vaccinated with Pfizer/BioNTech (BNT162b2) (n = 26), Moderna (mRNA-1273) (n = 21), Janssen (Ad26.COV2.S) (n = 1), or unvaccinated with no known exposure to COVID-19 (n = 2) (Table 2). Samples were collected at various timepoints post-vaccination, ranging from 1 month post-primary vaccination (2 doses) to 3 months after the fourth dose (primary vaccination + 2 booster doses). The age of study volunteers ranged from 23 to 65 years (Median = 45) (Females n = 28, Males n = 22) (Table 2).

To evaluate the effect of collection tube types on antibody results, blood was collected from each of the donors into plastic (BD, Cat# 367820), glass (BD, Cat# 366430), and gel separator tubes (BD, Cat# 367988). To evaluate the effect of anticoagulants on serology results, blood from each donor was drawn in tubes containing anticoagulants sodium heparin (Na Heparin; BD, Cat# 367874), sodium citrate (Na Citrate; BD, Cat# 363083), acid citrate dextrose (ACD; BD, Cat# 364606), or ethylenediaminetetraacetic acid (EDTA; BD, Cat# 366643) (Table 1).

5.2. Sample preparation

Whole blood was processed to obtain serum and plasma components, which were then placed in appropriate vials and labeled. For serum, the blood was allowed to clot for 30–60 min (min) prior to centrifugation at 1300×*g* for 20 minutes at 20–25 °C to separate the serum from the cells. The subsequent components of the whole blood were placed in the appropriately labeled containers as either serum or plasma samples and shipped on dry ice to the Vaccine, Immunity, and Cancer Directorate (VICD) at the Advanced Researched

Table 2
Demographic data.

	Unvaccinated		Moderna mRNA-1273		Janssen Ad26.COV2. S		Pfizer/BioNTech BNT162b2	
Sex	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Female	1	33	11	52	1	100	15	59
Male	1	33	10	48	0	0	11	41
Total	**2**	**100**	**21**	**100**	**1**	**100**	**26**	**100**

Technology Facility (ATRF) in Frederick, MD. The serum and plasma samples were stored at −80 °C until the time of testing. The samples were thawed on wet ice, divided into smaller aliquots, and subjected to heat inactivation at 56 °C for 30–60 min prior to testing.

5.3. Enzyme-linked immunosorbent assay (ELISA)

ELISAs were conducted to measure IgG antibodies against the SARS-CoV-2 Spike protein in human serum and plasma, as previously described [12]. Coated Maxisorp 96-well plates were incubated at 4 °C for at least 24 h. Following coating, the plates were washed with a PBS-Tween buffer and blocked using a mixture of PBS-Tween 0.2 % and 4 % skim milk for 90 min.

Heat-inactivated samples were subjected to appropriate in-well dilution series. Plates underwent a 60-min incubation at room temperature (RT). Next, the plates were washed and incubated with a goat anti-human IgG enzyme horseradish peroxidase (HRP)-conjugate for 60 min.

Following an additional wash, the plates were developed with 3,3′,5,5′-tetramethylbenzidine (TMB) to initiate a colorimetric change. The plates were incubated for 25 min, and the reaction was terminated using 0.36 N sulfuric acid.

The ELISA assays were read at 450_{nm} and 620_{nm} using a SpectraMax plate reader (Molecular Devices). Data analysis was performed using SoftMax Pro GxP 7.0.3. The quantification of IgG was reported as binding antibody units per milliliter (BAU/mL).

5.4. Avidity enzyme-linked immunosorbent assays (chaotrope ELISA)

Avidity ELISA assays, also known as chaotrope ELISA, were conducted to assess the binding strength of IgG antibodies against the SARS-CoV-2 Spike protein, as previously described [12]. The avidity ELISA assays were performed using serum and plasma samples appropriately diluted in assay buffer to produce optical densities (OD) ranging from 0.5 to 1.3 OD units at 450_{nm}.

Each assay plate tested 5 serum or plasma samples in duplicate. After incubating each sample on the assay plate at RT for 1 h, the plates were washed and then incubated with various dilutions of urea (ranging from 0 to 7 Molar [8]) for 15 min at RT. The procedure thereafter followed the same as the ELISA, as described above. Serum and plasma avidity data were reported as avidity indices, representing the Molar (M) concentration of urea required to reduce the optical density of the sample to 80 % (AI_{80}) of untreated wells.

5.5. Luminex bead-based multiplex serology assay

Multiplex assays were utilized to simultaneously quantify antibody responses against 11 different proteins, as previously described (Table S1) [13]. Assay cutoff concentrations for the individual proteins can be found in Table S2 [13]. Heat-inactivated samples, standards, and controls were subjected to appropriate in-well dilution series in Dulbecco's Phosphate-Buffered Saline (DPBS) with 1 % Bovine Serum Albumin (BSA). Samples were then loaded onto nonbinding surface (NBS) flat bottom 96-well plates, followed by the addition of the capture protein solution containing the 11 magnetically coupled proteins (~40,000 beads/mL).

The plates were sealed with a light-blocking plate sealer and incubated at RT while shaking at 750 rpm for 30 ± 5 min. Subsequently, the plates were washed with a PBS-Tween buffer using a plate washer equipped with a magnetic adapter to secure the coupled beads during washing. After washing, the goat anti-human IgG (γ chain specific) R-phycoerythrin (PE) conjugate was added, and the plates were sealed and shaken at 750 rpm at RT for an additional 30 ± 5 min. Following another wash step the sealed plates were incubated at RT while shaking (750 rpm) for a minimum of 10 min to assure homogenous dispersion of magnetic beads.

The Phycoerythrin (PE) - conjugated streptavidin signal emitted by the coupled beads was measured using the Luminex dual-laser detection instrument, FLEXMAP 3D, with xPONENT 4.3 software, following the assigned protocols to determine the target protein concentration present in the samples.

SARS-CoV-2 S protein RBD constructs were developed by Mount Sinai (Icahn School of Medicine at Mt. Sinai, New York, NY) and designated with an "M" in the protein name or by the Ragone Institute of Massachusetts General Hospital (MGH), Massachusetts Institute of Technology (MIT), and Harvard (Boston, MA) and designated with an "R" in the protein name; these constructs were used for RBD WT and variant protein constructions [13]. These proteins, CoV2_S, CoV2_N, R_RBD, R_RBD_UK, R_RBD_E484K, M_RBD, M_RBD_UK, M_RBD_SA, M_RBD_E484K, CoV1_S, and MERS_S, are summarized in Table S1.

5.6. Statistical analysis

Data analysis was performed using R version 4.3.1 with RStudio 2023.06.1 Build 524. Given the limited sample size for both sample collection tube type ($n = 21$) and different anticoagulants ($n = 29$), and the presence of more than 2 comparison groups, the non-parametric Friedman test was performed to assess differences in the distribution of the results by collection tube type or anticoagulant. In instances where statistically significant differences were found ($\alpha = 0.05$, $p < 0.05$), post-hoc analyses were executed to determine the statistical significance of individual comparisons. The pairwise Wilcoxon signed-rank test was utilized for this purpose, with Bonferroni correction applied to mitigate the compounding risk for Type I errors (inappropriate rejection of the null hypothesis) that could occur when performing multiple comparisons within the same dataset. The null hypothesis in the case of this study would be that there is no significant difference between container physical characteristics (serum samples) or anticoagulant presence (plasma samples).

A Spearman correlation analysis was used to verify that as one sample increased in concentration of antibody or avidity the correlating donor sample with the different container parameters also increased, verifying that the samples being compared were from

the same source sample. This method was chosen due to the non-normal distribution of the data, and the Spearman correlation coefficient (ρ) was used to determine the strength of the associations between the parameters and all correlations with a p-value <0.05 were significant [14]. A value of $\rho > 0.70$ was considered to indicate a strong relationship [14].

5.7. Data adjustments

Upon investigating the vendor-provided information on the anticoagulant tubes, it was observed that both the ACD and sodium citrate tubes contained a liquid volume of their respective anticoagulants, as opposed to a coating or powder found in the EDTA and sodium heparin tube. Assuming the standard volume of a full blood draw was collected, this additional volume in the tubes resulted in an approximate dilution of the sample by 15 % and 10 % for ACD and sodium citrate, respectively, prior to assay dilution.

To address this, a re-analysis was conducted, taking into consideration the increased dilution in the ACD and sodium citrate samples. The mean antibody concentration (BAU/mL) for ACD was adjusted by a factor of 1.15 to account for the new dilution, while the sodium citrate mean antibody concentrations were adjusted by a factor of 1.10. This resulted in updated mean antibody concentrations for the anti-SARS-CoV-2 Spike IgG ELISA and all Luminex Assays for subsequent statistical re-analysis.

While analyzing the anti-SARS-CoV-2 Spike IgG Avidity assay, noticeable differences emerged between plasma and serum samples from the same donor. This observation prompted a re-analysis specifically focusing on the plasma samples, excluding the serum matrix from the dataset entirely.

Funding

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Ethics statement

Informed consent was obtained from the donors that their samples will be used for laboratory experimentation as described in this technical optimization study. The archived human samples were collected by Occupational Health Services (OHS), National Cancer Institute (NCI) Frederick, Frederick, MD, under the Research Donor Protocol OH99CN046.

Human specimens used by the Vaccine, Immunity, and Cancer Directorate (VICD) in this research were obtained via the NCI Frederick Research Donor Program (RDP). A primary function of the RDP is to support the development and standardization of biological assays by provision of specimens collected from healthy volunteers.

Data availability statement

Data will be made available upon request.

Author declarations

The authors declare no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nicholas C. Castro: Writing – original draft, Methodology, Investigation, Data curation. **Jimmie Bullock:** Supervision, Project administration, Methodology. **Katarzyna Haynesworth:** Supervision, Project administration. **Sarah Loftus:** Methodology, Investigation. **Jordan Metz:** Methodology, Investigation. **Hayley North:** Visualization, Formal analysis. **Troy J. Kemp:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Ligia A. Pinto:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ligia Pinto reports financial support was provided by National Cancer Institute. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the Occupational Health Services (OHS), National Cancer Institute (NCI) Frederick, Frederick, MD and the blood donors who made this research possible. We would also like to thank Heidi Hempel (Ph.D.) for her assistance in writing

and editing this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34449.

References

[1] W.J. Wiersinga, A. Rhodes, A.C. Cheng, S.J. Peacock, H.C. Prescott, Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review, JAMA 324 (2020) 782–793, https://doi.org/10.1001/jama.2020.12839.

[2] M.P. Cheng, J. Papenburg, M. Desjardins, S. Kanjilal, C. Quach, M. Libman, S. Dittrich, C.P. Yansouni, Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2: a narrative review, Ann. Intern. Med. 172 (2020) 726–734, https://doi.org/10.7326/M20-1301.

[3] R. Verbeke, I. Lentacker, S.C. De Smedt, H. Dewitte, The dawn of mRNA vaccines: the COVID-19 case, J. Contr. Release 333 (2021) 511–520, https://doi.org/10.1016/j.jconrel.2021.03.043.

[4] X. He, J. Su, Y. Ma, W. Zhang, S. Tang, A comprehensive analysis of the efficacy and effectiveness of COVID-19 vaccines, Front. Immunol. 13 (2022) 945930, https://doi.org/10.3389/fimmu.2022.945930.

[5] A. Bastin, S. Fooladi, A.H. Doustimotlagh, S. Vakili, A.H. Aminizadeh, S. Faramarz, H. Shiri, M.H. Nematollahi, A comparative study on the effect of blood collection tubes on stress oxidative markers, PLoS One 17 (2022) e0266567, https://doi.org/10.1371/journal.pone.0266567.

[6] A.A. Rivolta, D.C. Pittman, A.J. Kappes, R.K. Stancil, C. Kogan, M.G. Sanz, The type of anticoagulant used for plasma collection affects in vitro Rhodococcus equi assays, BMC Res. Notes 15 (2022) 50, https://doi.org/10.1186/s13104-022-05933-4.

[7] A. Vignoli, L. Tenori, C. Morsiani, P. Turano, M. Capri, C. Luchinat, Serum or plasma (and which plasma), that is the question, J. Proteome Res. 21 (2022) 1061–1072, https://doi.org/10.1021/acs.jproteome.1c00935.

[8] J. Sotelo-Orozco, S.Y. Chen, I. Hertz-Picciotto, C.M. Slupsky, A comparison of serum and plasma blood collection tubes for the integration of epidemiological and metabolomics data, Front. Mol. Biosci. 8 (2021) 682134, https://doi.org/10.3389/fmolb.2021.682134.

[9] R.A. Bowen, A. Sattayapiwat, V. Gounden, A.T. Remaley, Blood collection tube-related alterations in analyte concentrations in quality control material and serum specimens, Clin. Biochem. 47 (2014) 150–157, https://doi.org/10.1016/j.clinbiochem.2013.11.003.

[10] E.M. Smets, J.E. Dijkstra-Lagemaat, M.A. Blankenstein, Influence of blood collection in plastic vs. glass evacuated serum-separator tubes on hormone and tumour marker levels, Clin. Chem. Lab. Med. 42 (2004) 435–439, https://doi.org/10.1515/cclm.2004.076.

[11] R.A.R. Bowen, G.L. Hortin, G. Csako, O.H. Otañez, A.T. Remaley, Impact of blood collection devices on clinical chemistry assays, Clin. Biochem. 43 (2010) 4–25, https://doi.org/10.1016/j.clinbiochem.2009.10.001.

[12] T.E. Hickey, T.J. Kemp, J. Bullock, A. Bouk, J. Metz, A. Neish, J. Cherry, D.R. Lowy, L.A. Pinto, SARS-CoV-2 IgG Spike antibody levels and avidity in natural infection or following vaccination with mRNA-1273 or BNT162b2 vaccines, Hum. Vaccines Immunother. 19 (2023) 2215677, https://doi.org/10.1080/21645515.2023.2215677.

[13] D.R. Roy, T.J. Kemp, K. Haynesworth, S.A. Loftus, L.A. Pinto, Development, validation, and utilization of a luminex-based SARS-CoV-2 multiplex serology assay, Microbiol. Spectr. 11 (2023) e0389822, https://doi.org/10.1128/spectrum.03898-22.

[14] P. Schober, C. Boer, L.A. Schwarte, Correlation coefficients: appropriate use and interpretation, Anesth. Analg. 126 (2018) 1763–1768, https://doi.org/10.1213/ANE.0000000000002864.