Oregon Health & Science University
School of Medicine

Scholarly Projects Final Report

Title *(Must match poster title; include key words in the title to improve electronic search capabilities.)*

Performance of Dry Blood Spot Samples to Measure SARS-CoV-2 Spike Protein Antibodies in a Vaccinated Cohort

Student Investigator's Name

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Project Course *(Indicate whether the project was conducted in the Scholarly Projects Curriculum; Physician Scientist Experience; Combined Degree Program [MD/MPH, MD/PhD]; or other course.)*

Scholarly Projects Curriculum

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Infectious Disease

Scholarly Project Final Report

Concentration Lead's Name

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Project/Research Question

Can dry blood spot (DBS) testing be used to accurately estimate post-vaccination SARS-CoV-2 spike protein antibody levels?

Type of Project *(Best description of your project; e.g., research study, quality improvement project, engineering project, etc.)*

Research study

Key words *(4-10 words describing key aspects of your project)*

Dry blood spots (DBS), SARS-CoV-2 antibodies, COVID-19 pandemic, indirect enzyme-linked immunoassay (ELISA), RBD spike protein, Pfizer mRNA vaccine

Meeting Presentations

If your project was presented at a meeting besides the OHSU Capstone, please provide the meeting(s) name, location, date, and presentation format below (poster vs. podium presentation or other).

N/A

Publications *(Abstract, article, other)*

If your project was published, please provide reference(s) below in JAMA style.

Manuscript in progress.

Submission to Archive

Final reports will be archived in a central library to benefit other students and colleagues. Describe any restrictions below (e.g., hold until publication of article on a specific date).

N/A

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Next Steps

What are possible next steps that would build upon the results of this project? Could any data or tools resulting from the project have the potential to be used to answer new research questions by future medical students?

-Determine the sensitivity and specificity of the assay utilizing DBS samples
-Create a ROC curve
-Optimize assay for DBS-derived antibody detection & develop DBS card sampling method (postal services, utilize in low resource settings such as in Thailand)
-Use DBS technology for other viruses (Dengue)

Please follow the link below and complete the archival process for your Project in addition to submitting your final report.

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Student's Signature/Date *(Electronic signatures on this form are acceptable.)*
This report describes work that I conducted in the Scholarly Projects Curriculum or alternative academic program at the OHSU School of Medicine. By typing my signature below, I attest to its authenticity and originality and agree to submit it to the Archive.

x  Francesca (Francie) Goodstein 3/13/2022

Student's full name

Mentor's Approval *(Signature/date)*

x 

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Mentor Name

Scholarly Project Final Report

Report:

Introduction

Dry blood spot (DBS) testing is a form of noninvasive sampling where a few drops of blood are blotted and dried on filter paper cards. The specimens can be stored in plastic bags, are stable without refrigeration and can be shipped in the mail to laboratories for processing.[1-3] DBS samples can be used to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein antibodies and may offer an alternative for serologic testing of vaccinated populations.[4] The utilization of DBS samples to measure antibody levels in subjects vaccinated with a SARS-CoV-2 mRNA vaccine has never been investigated in the literature and may offer a less invasive, more accessible and resource-sparing method for seroprevalence studies of large populations during the COVID-19 pandemic.

Periodically measuring antibody titers in vaccinated individuals is currently achieved by collecting serum samples via venipuncture which requires trained personnel and patients to come into a lab in person. Developing a validated method for measuring antibody titers using DBS samples could make this process much more practical and stream-lined for both patients and research personnel. Optimizing the use of DBS samples could be a game changer for how humoral immunity of vaccinated patients are followed over time, and for gaining information on a large scale that could influence future vaccination protocols. DBS sampling introduces the opportunity for surveillance of humoral immunity and disease burden in large and varied populations all over the world.

The question this research project is trying to answer is whether dry blood spot samples can be used as a reliable and valid tool to accurately estimate post-vaccination SARS-CoV-2 spike protein antibody levels using indirect enzyme-linked immunoassay (ELISA) when compared to serum samples. The study design is a prospective randomized cohort study utilizing matched DBS and serum samples from a SARS-CoV-2 Pfizer vaccine cohort at OHSU in 2020-2021

Methods

Sample Collection

We collected blood samples from 177 volunteers in the COVID-19 vaccinated cohort at OHSU by venipuncture 14 days after receiving the second dose of the Pfizer mRNA vaccine. At the time of sample collection, 60-70µL of blood was blotted on to each circle of a DBS filter paper card using a pipette. The serum samples were processed and aliquoted in the laboratory, and the DBS cards were dried. All samples were deidentified from PHI and stored at -20 °C while in use for the study.

Reconstitution of Sample From DBS

One circle of dried blood on a filter paper was punched into a well on a 96-well plate using a 3.2-mm device puncher. 75µL of Phosphate-Buffered Saline + Tween 20 (PBS-T) was added to each DBS well and was incubated overnight at room temperature on a shaker plate. The plates were centrifuged at 1,500 g for 10 minutes to pellet any debris. The DBS eluate was taken directly from the wells to be used for ELISA assays as described below.

Measurement of SARS-CoV-2-antibodies: Indirect ELISA

The DBS and serum samples were tested using SARS-CoV-2 IgG indirect ELISA following a well-established protocol optimized in Dr. Bill Messer's lab. In brief, 96-well plates were coated with 100 µL of 0.5 µg/mL of purified receptor binding domain (RBD) of the spike protein + Phosphate-Buffered Saline (PBS). Plates and

diluted samples were blocked with 5% non-fat dry milk (NFDM) + PBS-T at starting dilutions of 1:4 DBS eluate and 1:100 serum, with 4-fold serial dilutions. Donkey polyclonal anti-human horseradish peroxidase conjugated antibodies were diluted 1:3000 in 5% NFDM + PBS-T. Plates were washed with PBS-T in between blocking and secondary antibody incubation steps. Plates were developed with OPD substrate and stopped with 1M HCl. The absorbance of each well was read at 492 nm using a microplate reader. All assays were performed in duplicate with appropriate positive and negative controls (positive control: convalescent plasma; negative controls: naïve serum and DBS).

Data Processing & Statistical Analysis

Demographic data associated with each sample was collected and the time of sample collection. Absorbance of each well was collected at the time of assay, and all the samples were normalized to controls. Prism was used to examine the data by creating a scatterplot with a trend line and the *r* value was calculated. Prism was also utilized to create a Bland-Altman plot.

Results

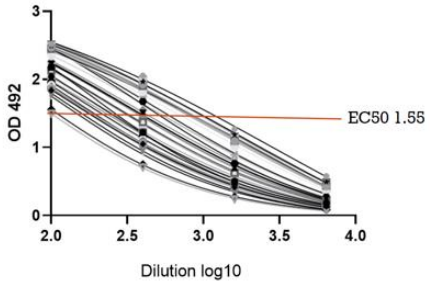
In this study, 177 matched serum and DBS samples were collected and processed from 177 volunteers in the SARS-CoV-2 vaccine cohort 14 days after receiving the second dose of the Pfizer spike protein mRNA vaccine. The demographic characteristics are described in Table 1. Of the 177 volunteers, 21% (37) were male, and 79% (140) were female. The average age of the volunteers is 45.5 years old, with a median age of 43 years.

Table 1: Demographic Characteristics of Population

Gender		
	Male (percent)	37 (21%)
	Female (percent)	140 (79%)
	Total	177
Age (years)		
	Mean (SD)	45.5 (13.4)
	Median (min, max)	43 (23, 74)

We quantified SARS-CoV-2 antibody concentrations from 177 matched serum (diluted 1:100) and DBS eluate (diluted 1:4) samples. The EC50 of each sample was determined by the concentration of antibody at OD_{492} of 1.55 by generating titration curves in Prism. An example of an ELISA standard curve of a subset of serum samples with EC50 values indicated by the red line is shown in Figure 1. The concentrations of duplicate samples were averaged for analysis. Correlates of variation between intra and interpolate controls and between duplicate samples were maintained below 25% to ensure precision of the assay.

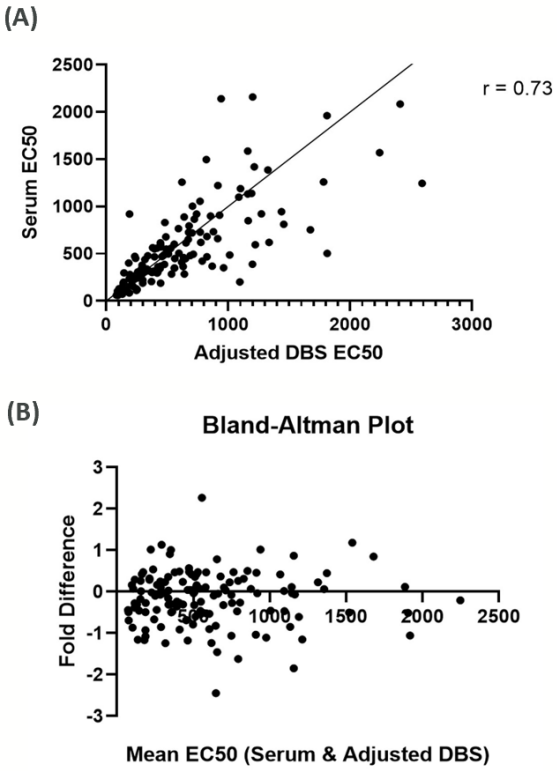
Figure 1: ELISA standard curves of subset of serum samples



The ELISA results using serum showed a higher signal than DBS samples. We observed a nearly 37-fold reduction in mean antibody concentration in DBS eluate compared with matched serum. We plotted the 177 matched serum/DBS SARS-CoV-2 IgG ELISA results and fitted an exponential trend line (Figure 2A). The DBS concentrations were adjusted by a multiplicative value of 36.9. The *r* value was 0.73, indicating a moderate yet significant correlation. We created a Bland-Altman plot to access the agreement between DBS and serum ELISA results. The Bland-Altman plot in Figure 2B shows minimal differences in the majority

of the results observed by sample type (95% CI -1.16 to 0.87). Preliminary results from 37 matched DBS and serum samples of a healthy cohort with confirmed negative SARS-CoV-2 infection and pre-vaccine show that there is light cross-reactivity below the limit of detection.

Figure 2: Effectiveness of DBS sampling for SARS-CoV-2 anti-RBD spike protein detection.
(A) Correlation between matched DBS eluate (1:4) and serum (1:100) ELISA EC50 results with trend line and R-value. DBS EC50 values adjusted to serum EC50s (average adjustment factor of 36.9). (B) Bland-Altman mean-difference comparison of DBS eluate and serum ELISA EC50 results. 95% confidence interval [-1.16 to 0.87].



Discussion

This study is the first known attempt to measure SARS-CoV-2 antibody levels from DBS samples in subjects vaccinated with a SARS-CoV-2 spike protein mRNA vaccine. The primary endpoint of this study was the performance of DBS compared with serum samples for determining SARS-CoV-2 spike protein antibody titers. Our preliminary results show that there is a statistically significant correlation ($r > 0.7$) between DBS and serum sample SARS-CoV-2 IgG antibody levels in subjects vaccinated against the novel coronavirus after two doses of the Pfizer mRNA spike protein vaccine (Figure 2A). The Bland-Altman plot shows that there is minimal bias between the sampling methods (Figure 2B). This study suggests that DBS samples can be used for the detection of SARS-CoV-2-specific antibodies status post two doses of Pfizer vaccine with results comparable to serum samples.

While the correlation of antibody levels between DBS and serum samples is not as high as in a previous SARS-CoV-2 study conducted by the CDC[4], the results presented in this study are preliminary and statistically significant. This study has several limitations. Firstly, the limitation of time was a major factor.

Further optimization of the indirect ELISA assay, re-running of samples to achieve a lower correlation of variation (ideally <15%), and more in-depth data analysis were not possible prior to the end of this study period. The time limitation also made it not possible to complete all the data analysis for the health subject cohort, and so we have yet to determine the sensitivity and the specificity of the ELISA assay for use of DBS samples for quantifying antibody titers status post vaccination.

Secondly, DBS samples were not collected in the way that they would be intended to be collected in a real-life scenario. Blood that had been collected by skilled phlebotomists was blotted directly onto filter paper cards with a pipette with a controlled volume. DBS samples are intended to be collected independently by the patient/subject without a phlebotomist by pricking of the finger with a lancet resulting in blood volume that could vary drastically between each sample. In addition, the cards used in this study were immediately dried and stored in a freezer in the laboratory rather than being mailed or stored in room temperature for an extended period. A crucial next step of this study would be to determine the performance characteristics of this assay when using DBS cards with sample provided by the patient themselves with a lancet.

In summary, this study suggests that DBS samples can be used to measure SARS-CoV-2 antibodies with levels comparable to serum samples from a vaccinated cohort. Further optimization of the antibody assay along with development of a DBS sample collection method not requiring venipuncture can lead to a less invasive, more accessible, and resource-sparing method for seroprevalence studies of large vaccinated populations during the COVID-19 pandemic.

Conclusions

There is a moderate correlation (r = 0.73) between matched serum and DBS samples in detecting SARS-CoV-2 antibody levels in a cohort of patients after two doses of the Pfizer mRNA vaccine, and there are minimal differences in results observed by sample type. These preliminary results suggest that DBS cards can be used as a reliable sampling method comparable to venipuncture for measuring SARS-CoV-2 spike protein antibodies status post vaccination.

References *(JAMA style format)*

1. Grüner, N., O. Stambouli, and R.S. Ross, Dried blood spots--preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp, 2015(97).

2. Page, M., et al., Are all blood-based postal sampling kits the same? A comparative service evaluation of the performance of dried blood spot and mini tube sample collection systems for postal HIV and syphilis testing. Sex Transm Infect, 2021. 97(3): p. 209-214.

3. van Loo, I.H.M., et al., Screening for HIV, hepatitis B and syphilis on dried blood spots: A promising method to better reach hidden high-risk populations with self-collected sampling. PLoS One, 2017. 12(10): p. e0186722.

4. Morley, G.L., et al., Sensitive Detection of SARS-CoV-2-Specific Antibodies in Dried Blood Spot Samples. Emerg Infect Dis, 2020. 26(12): p. 2970-2973.