Application of Volumetric Absorptive Microsampling Devices to the Simoa® SARS-CoV-2 IgG Antibody Test

Introduction

Dried blood spots (DBS) have many advantages over traditional sample matrices such as serum, plasma, and liquid whole blood. Chief among these advantages is simplified sample collection, storage, and shipment.[2,3] DBS from finger sticks can be collected and shipped without freezing or cold storage, eliminating the need for a centrifuge, freezers, or even a phlebotomist.[1] Ultimately, DBS enables decentralized sample collection, whether in areas lacking laboratory infrastructure, long term studies that struggle with patient retention, or monitoring of chronic diseases. [4,5]

Dried blood spots do however have major challenges in collection, processing, and bioanalysis. The major drawback being the variability in the amount of blood collected. The volume of blood collected on a spot can be quite variable and challenging to obtain from individuals such as small children. [6]

Volumetric absorptive microsampling (VAMS) devices, such as the Neoteryx® Mitra®, address many of these challenges. The VAMS devices absorb volumes with a high degree of accuracy. The tips lend themselves to automation and improve on the carryover that may be observed with punching out spots from traditional cards.

Here we employ VAMS devices as a proof of concept with the Simoa® SARS-CoV-2 IgG Antibody Test. The devices could be useful for screening populations for the presence of anti SARS-CoV-2 IgG antibodies by allowing decentralized collection at home. This would enable rapid testing by reaching greater quantities of people quickly without creating additional exposure risk to the coronavirus by going to a hospital for sample collection.

Since the analyte of interest is not present in uninfected normal hosts, the feasibility experiments described here should be done with COVID positive finger stick samples. Due to the limited resources and availability of such samples, spiked venous whole blood was used to determine feasibility.

In this study, venous whole blood was drawn, spiked with SAR-CoV-2 IgG, and applied onto VAMS tips as a surrogate matrix to finger stick blood.

Materials and Methods

Figure 1 illustrates the blood sample collection and processing in this work. 20 µL Neoteryx® Mitra®, VAMS devices samples were evaluated for extraction method, correlation to plasma, device repeatability, sample stability, and freeze-thaw stability. Table 1 outlines the experimental setup for each study.

1. 21 presumable healthy donors were recruited for this work. Venous whole blood samples (K2 EDTA tube, BD, NJ) were obtained for each.
2. VAMS devices with collected sample were packaged in the original clamshell, sealed in a light-protected pouch with desiccants at room temperature (RT) for

COVID 19

24 hours, and then stored at -80°C until time of extraction.

3. Prior to applying the whole blood to the VAMS device, the blood was spiked with either chimeric antibody (Sino Biological, China) or plasma containing endogenous antibody (COVID positive plasma with high SARS-CoV-2 IgG concentrations) (BioIVT, NY) into 0.5 mL of venous whole blood.
4. An aliquot of spiked venous whole blood was saved for testing in a Hemoglobin Colorimetric Assay (AbCam, MA). The hemoglobin test was used to determine the hematocrit of extracted dried blood and whole blood prior to plasma preparation.
5. Spiked venous whole blood drops (30 µL per drop) were pipetted onto a parafilm strip and then absorbed into a VAMS device. The remaining spiked whole blood was processed into plasma by centrifugation (3000 rpm, 4°C, 12 min).
6. VAMS tips were packaged back into the original clamshell and placed in a sealed pouch with desiccant at RT for 24 hours (or longer for stability study) and then stored at -80°C.
7. Extraction from VAMS devices used a PBS-based buffer (200 µL).
8. Extracted material was used in experiments to determine the Extraction Efficiency, Correlation to Plasma, tip to tip Repeatability, and Hemoglobin tests or stored at -80°C at the designated stability time points. Fresh, unfrozen extracts were used for the freeze-thaw evaluations.
9. The extracts and corresponding plasma were analyzed using the Simoa® SARS-CoV-2 IgG Antibody Test (Quanterix, MA). Extractions were further diluted 100x (10x was already performed during step 7) with sample diluent. Spiked plasma was diluted 1000x with sample diluent as per the kit MRD.
10. Samples were processed and analyzed using the Simoa HD-X analyzer. All calibrators, controls and samples were run in duplicates according to the assay kit instructions.

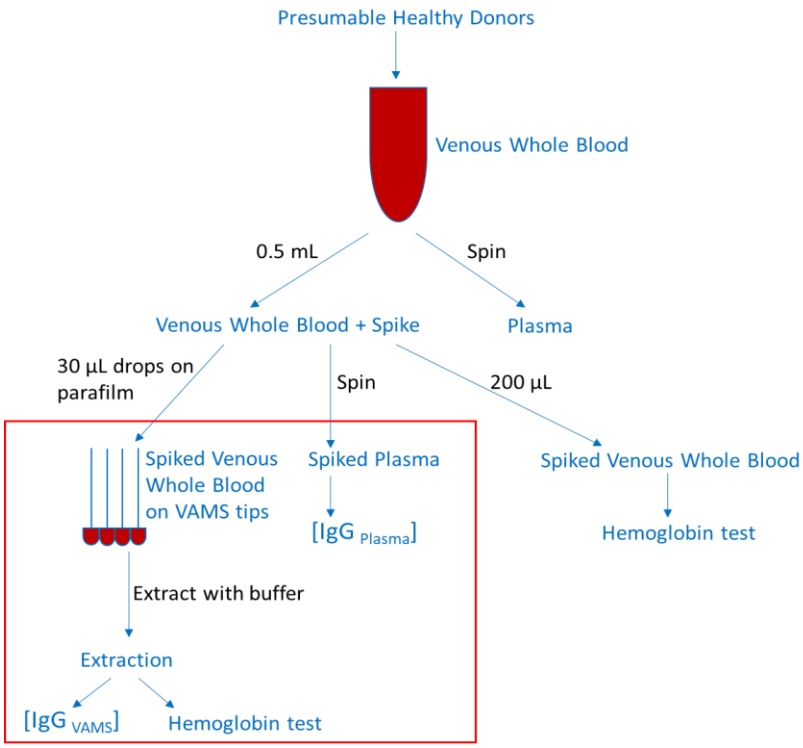


Figure 1. Blood sample collection and processing. Extraction and Spiked Plasma in red box were the two final products that tested for SARS-CoV-2 protein IgG, marked as [IgG_{VAMS}] and [IgG_{Plasma}] respectively.

Study	No. of Donors	Spike Level	Tests
Extraction	4	Spike all 4 donor samples with 10 µg/mL chimeric antibody	Extraction methods tested: • 2 hours at RT, 400 rpm • 4 hours at RT, 400 rpm • overnight at 4°C, 400 rpm • sonicate for 2 minutes (42 Hz)
Plasma Correlation	13	Spike 8 donor samples with either 50, 25, 10, 5, 2.5, 1, 0.5 or 0 µg/mL chimeric antibody Spike 5 donors with either 4, 2, 1, 0.5 or 0 µg/mL endogenous antibody	Correlation of SARS-CoV-2 protein IgG concentrations between spiked plasma samples and extractions
Device Repeatability	3*	Spike each of the 3 donor samples with 10, 1 and 0 µg/mL chimeric antibody	Evaluate tip to tip repeatability by %CV (4 tips per spike level per donor)
Stability	8*	Spike 2 donor samples with 10 µg/mL chimeric antibody, spike the remaining 2 donor samples with 4 µg/mL endogenous antibody	• One-month stability at RT (Time points: Day 1, 3, 7 and Week 2, 3, 4) • One-week stability at 37°C (Time points: Day 1, 4, 7 and 11)
Freeze-Thaw Stability	4*	Spike 2 donor samples with 10 µg/mL chimeric antibody, spike the remaining 2 donor samples with 4 µg/mL endogenous antibody	Evaluate for 3 Freeze-Thaw cycles

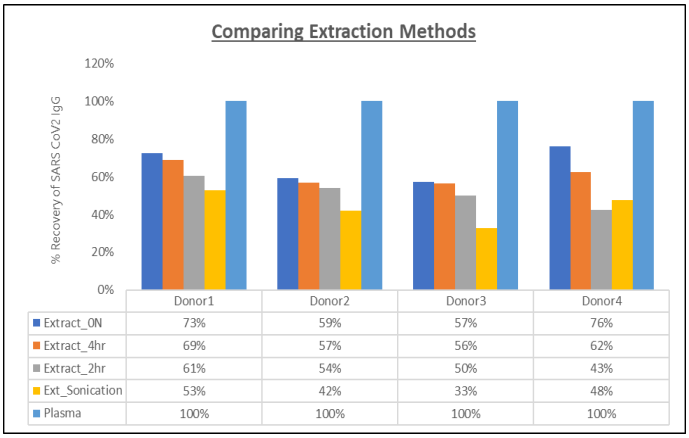
Table 1. Experimental setup for extraction, plasma correlation, device repeatability, stability, and freeze-thaw stability studies.

* Same donors were used as the Extraction study (4 out of 8 donors were the same for stability study).

Results

Extraction Study

Overnight extraction at 4°C, 400 rpm gave the highest recovery of SARS-CoV-2 protein IgG among tested extraction methods considering all donors, with 55% – 80% recovery observed (Figure 2). This agreed with the fact that the plasma volume was a fraction of the whole blood volume in VAMS tip samples, so that the [IgG_{VAMS}] would be lower than [IgG_{Plasma}]. The recovery of SARS-CoV-2 protein IgG with 4-hr, 2-hr at 30°C, 400 rpm and sonication extraction methods were not as efficient as overnight extraction. Therefore, overnight extraction was selected for the following validation work.



	Donor1	Donor2	Donor3	Donor4
Extract_ON	73%	59%	57%	76%
Extract_4hr	69%	57%	56%	62%
Extract_2hr	61%	54%	50%	43%
Ext_Sonication	53%	42%	33%	48%
Plasma	100%	100%	100%	100%

Figure 2. Comparisons of four different extraction methods with four donors. Matched plasma reading [IgG_{Plasma}] was marked as 100% for normalization. Percent recovery of different extraction methods was calculated by dividing the [IgG_{VAMS}] by corresponding [IgG_{Plasma}].

Plasma Correlation Study

Figure 3 illustrates the correlation of SARS-CoV-2 protein IgG concentrations between Neoteryx VAMS whole blood and EDTA Plasma. High correlation was observed between two sampling methods with R^2 = 0.9996 (Figure 3A). When hematocrit correction was applied to the [IgG $_{VAMS}$] (Figure 3B), the correlation slope was further improved from 0.5873 to 0.8217. Note: hematocrit was calculated as three times of the hemoglobin concentration and [IgG_{VAMS}] was then corrected for hematocrit to account for the volume difference between venous whole blood extractions and plasma samples. The above results suggest that the Neoteryx microsampling tip collection method is highly correlated with venous phlebotomy when assessing the SARS-CoV-2 protein IgG for individual human subjects.

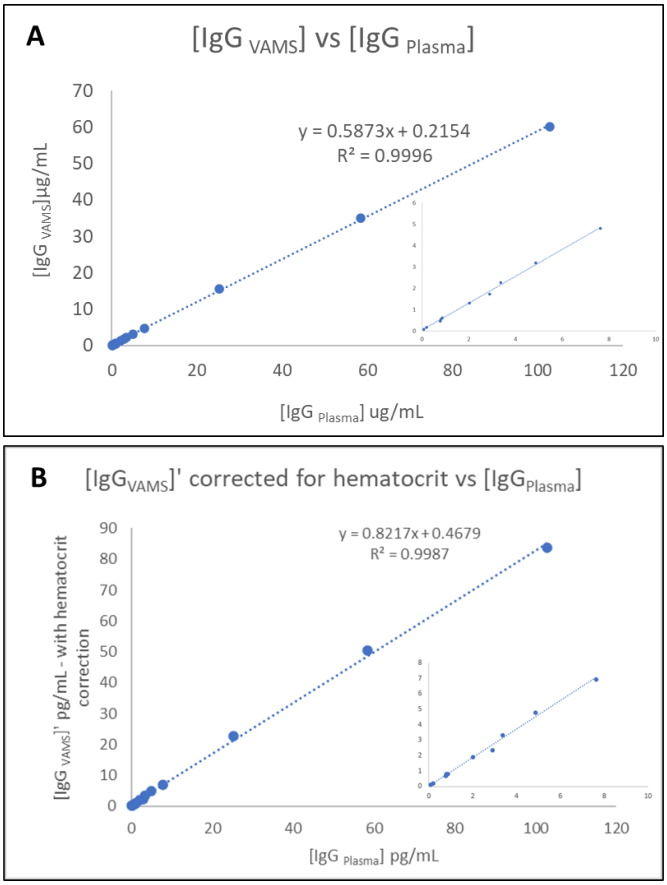


Figure 3. Correlation between Neoteryx VAMS whole blood and EDTA Plasma. A) [IgG_{VAMS}] was directly compared with [IgG_{Plasma}]. B) [IgG_{Plasma}]' corrected for Hematocrit was used for comparison. [IgG_{VAMS}]'=[IgG_{VAMS}]+ [IgG_{VAMS}]*HCT/100. HCT= [Hgb]*3

Repeatability Study

Figure 4 illustrates the grand CV of tip-to-tip [IgG $_{VAMS}$] within four tip replicates for each donor at three spiked levels. At low level (no spike, endogenous level), Donor 001 had concentrations close to the mid-spike of the other donors. Donor 002 and 003 were approximately 100x lower in concentration than Donor 001 for endogenous SARS-CoV-2 protein IgG. Donor 003 had the value less than the estimated LOQ therefore was excluded because no quantifiable data is available. Overall, the grand CV of tip-to-tip [IgG $_{VAMS}$] was less

than 12% which indicates good tip-to-tip repeatability (excluded the value < functional LLOQ).

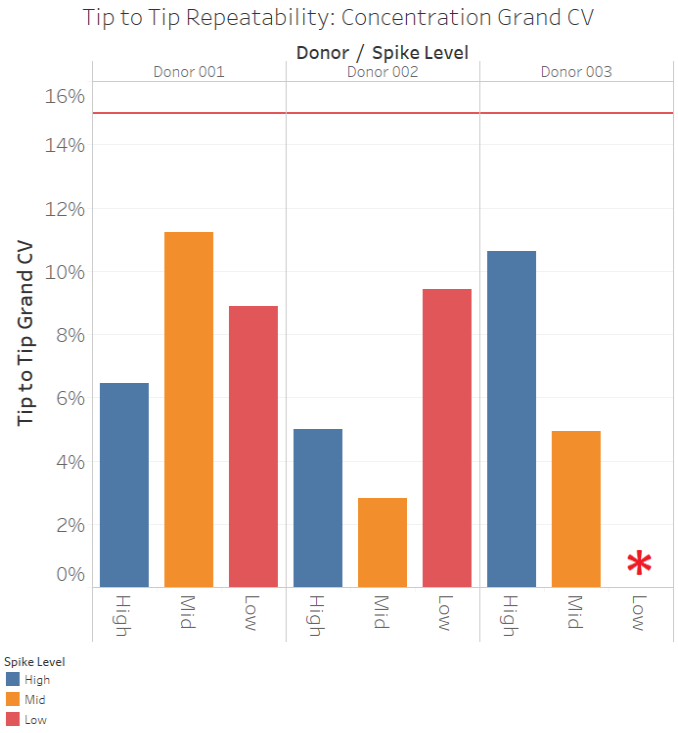


Figure 4. The grand CV of tip-to-tip [IgG $_{VAMS}$] within four tip replicates for each donor at three spiked levels.

Stability Study

Stability of dried blood VAMS tip samples were evaluated at both room temperature and 37°C. This study was aimed to address the stability during shipping and extreme environmental conditions during transportation. Figure 5 illustrates the [IgG $_{VAMS}$] % bias from Day 1 for up to one month (RT). Less than 20% change of SARS-CoV-2 protein IgG activity was observed at room temperature for 4 weeks. Donor 4 was slightly beyond the acceptable range but with a consistent trend. This result might be explained by a high CV for the readings on Day 1 skewing the expected value for subsequent time points. This suggests that the Neoteryx dried blood VAMS tip samples is stable for up to 1 month at room temperature.

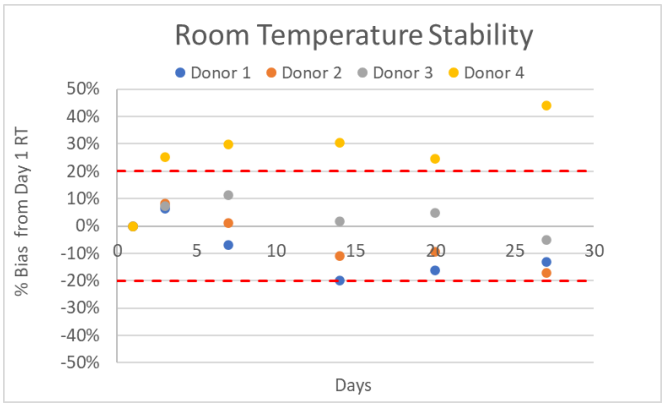


Figure 5. One-month stability at room temperature. SARS-CoV-2 protein IgG reading on Day 1 was normalized as 100%, the rest of the time points were calculated by dividing [IgG $_{VAMS}$] of each time point by [IgG $_{VAMS}$] of Day 1.

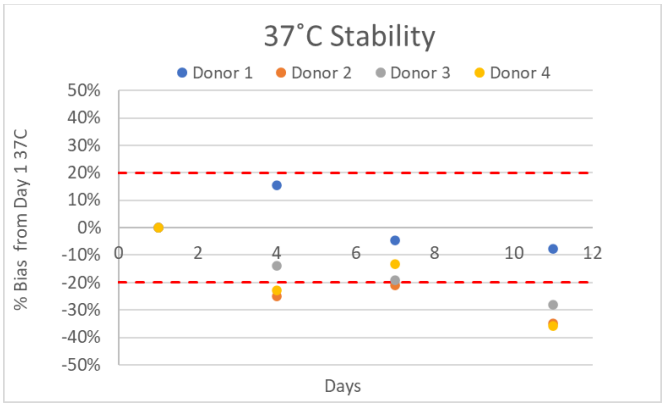


Figure 6. 11 days stability at 37°C. SARS-CoV-2 protein IgG reading on Day 1 was normalized as 100%, the rest of the time points were calculated by dividing [IgG $_{VAMS}$] of each time point by [IgG $_{VAMS}$] of Day 1.

Figure 6 illustrates the [IgG $_{VAMS}$] % bias from Day 1 for 11 days (37°C). Less than 20% decrease of SARS-CoV-2 protein IgG activity was observed at 37°C for 1 week. After storing VAMS tip samples for 11 days, the SARS-CoV-2 protein IgG activity for 3 out of 4 samples had a significant decrease. This suggests that the Neoteryx dried blood VAMS tip samples is stable under extreme environmental condition (37°C) during transportation for up to 1 week.

Freeze-Thaw Stability Study

Figure 7 illustrates the Freeze-Thaw (F-T) stability of dried blood VAMS tip samples for up to three freeze-

thaw cycles. No significant [IgG $_{VAMS}$] decrease was observed with fresh extractions, one F-T, two F-T and three F-T cycles. The average [IgG $_{VAMS}$] % bias from fresh extracts (no F-T) was within 20%. This suggests that the samples are stable for up to three freeze-thaw cycles.

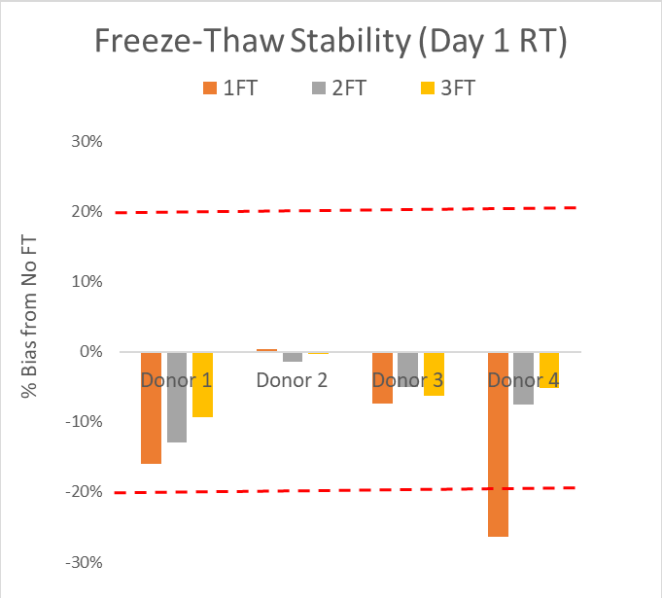


Figure 7. Freeze-thaw stability. [IgG $_{VAMS}$] % bias from fresh extracts was plotted as a function of the donors over 3 freeze-thaw cycles.

Conclusion

Neoteryx's volumetric absorptive microsampling (VAMS) proves to be a feasible method for measuring SARS-CoV-2 IgG by the Simoa® SARS-CoV-2 IgG Antibody Test. With excellent repeatability, correlation, and stability showed in initial results, this novel sample collection method could help mobilize a new chapter of large-scale sample testing that can be easily performed at home with minimal instructions. We anticipate these methods will be quickly adapted into mainstream COVID-19 testing and likely to be increasingly used for future applications.

Supplement

Extraction Method Comparison Study

To facilitate a large-scale study, Charles River Laboratories (CRL) was able to provide a more efficient sonication extraction method compared with Quanterix (Qtx)'s overnight extraction. CRL methods stated that "VAMS devices are placed into propriety buffer solution, plates are sonicated and once the elution is completed, sample is transferred to a sample plate for storage/testing." Four spiked VAMS tip samples were extracted with both Qtx and CRL methods (2 tips per method) and then tested using Simoa® SARS-CoV-2 IgG Antibody Test. Figure 8 illustrates the tip grand mean concentration comparison data between Qtx and CRL. No significant difference was observed between two extraction methods over five samples. This suggests that optimized sonication-based methods can be used as a replacement of the Qtx overnight method allowing for rapid sample preparation for large-scale testing.

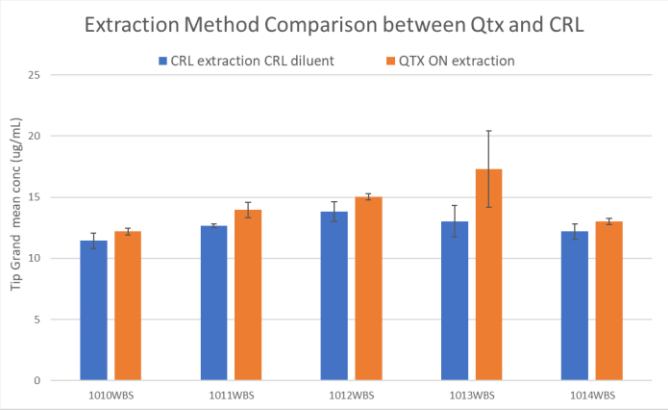


Figure 8. Extraction method comparison between Qtx and CRL. Qtx used overnight extraction at 4°C, 400 rpm. CRL used sonication method that takes less than 5 minutes.

References

1. Wang J, Li D, Wiltse A, Emo J, Jilchey SP and Zand MS. 2020. Application of volumetric absorptive microsampling (VAMS) to measure multidimentional anti-influenza IgG antibodies by the mPlex-Flu assay. Journal of Clinical and Translational Science. 3: 332-343.
2. Carlson, B.F., et al. "Dried Blood Spots: An Evaluation of Utility in the Field." Annals of Global Health, vol. 82, no. 3, 2016, p. 450., doi: 10.1016/j.aogh.2016.04.241.
3. Evans, Christopher, et al. "Implementing Dried Blood Spot Sampling for Clinical Pharmaco-kinetic Determinations: Considerations from the IQ Consortium Microsampling Working Group." The AAPS Journal, vol. 17, no. 2, 2014, pp. 292–300., doi:10.1208/s12248-014-9695-3.
4. Lim, Mark D. "Dried Blood Spots for Global Health Diagnostics and Surveillance: Opportunities and Challenges." The American Journal of Tropical Medicine and Hygiene, vol. 99, no. 2, 2018, pp. 256–265., doi:10.4269/ajtmh.17-0889.
5. Smit, Pieter W., et al. "Systematic Review of the Use of Dried Blood Spots for Monitoring HIV Viral Load and for Early Infant Diagnosis." PLoS ONE, vol. 9, no. 3, 2014, doi:10.1371/journal.pone.0086461.
6. Londhe, V., & Rajadhyaksha, M. (2020). Opportunities and obstacles for microsampling techniques in bioanalysis: Special focus on DBS and VAMS. Journal of Pharmaceutical and Biomedical Analysis, 182, 113102. doi:10.1016/j.jpba.2020.113102.

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