Development and Optimization of a Quantitative Assay for an Investigational Anti-Malarial Monoclonal Antibody Drug Candidate in Human Whole Blood Using Volumetric Absorptive Microsampling (VAMS)

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SUMMARY
Adaptation of volumetric absorptive microsampling (VAMS) for human whole blood collection into a bioanalytical laboratory workflow presents a significant advantage for patient populations affected by factors creating difficult blood collection (i.e. young children) over the traditional blood collection methods. VAMS addresses the challenges of sample volume, patient comfort, and offers a convenient, minimally invasive, and precise blood collection method. VAMS uses capillary action to absorb blood into a porous, hydrophilic tip at a specific volume. Dried samples can be stored and later extracted for analysis.

INTRODUCTION
In this investigation study, we focus on optimizing and implementing a quantitative ligand binding assay using VAMS-collected human whole blood containing an investigational human anti-malarial monoclonal antibody drug candidate. The VAMS device consists of a porous, hydrophilic tip that can absorb a specific volume of blood, available in 10, 20, and 30 µL. The VAMS tips are placed in a purple clamshell that snap closes on the tips, allowing for airflow through the device and protecting the loaded VAMS tips from contamination. The device is allowed to dry in a sealed storage bag with a desiccant (**Figure 1**). Key challenges include calibration and quality control preparation that are representative of samples, optimization of the drying time of the blood samples on the VAMS devices, drug candidate stability, ability to dilute samples with elevated drug concentration down to the range of analysis, and recovery of the drug candidate in the presence of possible interferences.



Figure 1. Storage bag, desiccant, and VAMS device.

METHOD
This pharmacokinetic ligand binding assay uses an MSD standard bind plate coated with the anti-drug antibody, blocked with blocker casein, followed by the extracted samples from the VAMS devices with a minimum required dilution (MRD) of 1:100 in a 10% mouse serum in DPBS + 1% bovine serum albumin (BSA) solution (MRD solution), and followed by the ruthenylated anti-drug antibody for detection.
Calibration and quality control samples were prepared by making a 20X concentration of the drug candidate in human serum, spiking into human whole blood with K2EDTA, and absorbed onto the VAMS devices. The calibration curve consists of nine points (10000, 7500, 6250, 5000, 2500, 1250, 625, 250, and 100 ng/mL) with 2 anchor points (20000 and 50.0 ng/mL). The QCs were made at 8000, 4000, and 300 ng/mL with the upper and lower quantitation (ULOQ, LLOQ) at 10,000 and 100 ng/mL, respectively. Samples were allowed to dry on the VAMS for 6-48 hours with a desiccant in a sealed bag. The dried blood samples on the VAMS devices were extracted 1:10 in a phosphate buffer solution containing tween-20 and BSA (extraction solution) shaking 16-24 hours at 2-8 °C at approximately 450 rpm. Prior to using the samples on the assay, the extracted samples are centrifuged at 500 x g for 2 minutes to remove any particulates that may be found from the VAMS tip. and further used in the pharmacokinetic assay (**Figure 2**).

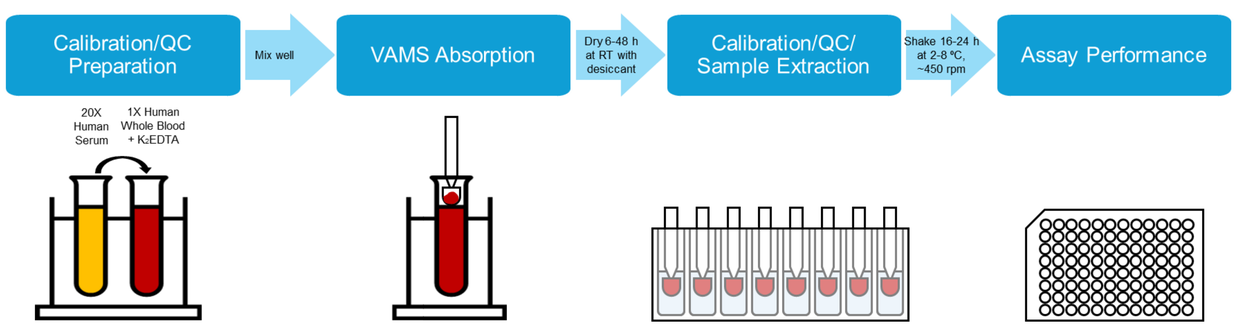


Figure 2. Sample preparation process of the calibration and quality control samples.

RESULTS
In developing the assay method, calibration and quality control samples needed to be prepared to represent the samples. Preparation of the drug candidate in human plasma with K2EDTA and human serum prior to spiking human whole blood were compared. It was observed that the samples prepared with human plasma occasionally contained red precipitate after the extracts were centrifuged at 500 x g for 2 minutes (**Figure 3**). The presence of the precipitate did not seem to impact on the results. The samples prepared in serum displayed less variability and used further in the assay development.
Accuracy and precision with calibration curve and QCs prepared with human serum were evaluated across 2 analysts with 3 sets of QCs/plate. The acceptance criteria for this parameter were ± 20.0% relative error (RE) and coefficient of variation (CV) for high, medium, and low QCs, and ± 25.0% RE and CV for ULOQ and LLOQ. Additionally, the total error must be within ± 30.0% for high, medium, and low QCs, and ± 40.0% for ULOQ and LLOQ. Using a 5-parameter fit with 1/Y2 weighting, the QCs, ULOQ, and LLOQ were within the acceptance criteria for intra-run and inter-run accuracy and precision with a total error ranging from 4.6-17.1%.
The drying process acts as a stabilizer for the analyte. VAMS dried at 2 hours provided variable results, as the samples were not fully dried. To evaluate an effective incubation range to dry the blood samples on the VAMS devices, samples were allowed to dry on the VAMS for 6, 8, 10, 12, 16, 18, 24, 48, and 96 hours in a desiccant-containing sealed bag at room temperature. Drying for 6-48 hours demonstrated stability by meeting the acceptance criteria of ± 20% RE of the drug concentration prepared. At 96 hours, the samples at 25 and 75 ng/mL of the drug were outside of the acceptance criteria (**Figure 4**).
Dilution linearity was explored to evaluate potential prozone and the ability to accurately dilute the drug candidate in the range of quantitation by preparing a high drug concentration sample on the VAMS device and investigating different matrices and sample diluent buffers. During this investigation, the calibration curve range was 12.5-3125 ng/mL. Ultra-high quality control (UHQC) at 500,000 ng/mL was tested under conditions A-D: (A) UHQC prepared with human plasma and spiked into human whole blood, dilution linearity samples prepared in human whole blood, and loaded onto the VAMS device; (B) UHQC prepared with human plasma and spiked into human whole blood, loaded onto the VAMS device, and dilution linearity samples prepared in extraction solution; (C) UHQC prepared with human plasma and spiked into human whole blood, loaded onto the VAMS device, and dilution linearity samples prepared in MRD solution; (D) UHQC prepared with human serum and spiked into human whole blood, loaded onto the VAMS device, and dilution linearity samples prepared in MRD solution (**Figure 5A**). Dilution linearity samples did not meet the acceptance criteria (± 25% RE at the range of quantitative) under conditions A, B, and C, but did meet the acceptance criteria under condition D (**Figure 5B**).
By diluting the samples in the MRD solution, an accurate dilution linearity was demonstrated while removing possible interferences seen in whole blood and acting as a blocking step. The drug candidate on the VAMS device was evaluated for dilution linearity and potential prozone effects with a calibration curve range of 100-10,000 ng/mL. Starting at a UHQC of 500,000 ng/mL, dilution linearity samples were within ± 25% RE at the range of quantitation (1:100, 1:500, and 1:5000). The maximum acceptable dilution was 1:5000 with no prozone effect observed (**Figure 6**).
Room temperature and up to 5 freeze-thaw stabilities of the extracted QCs were evaluated at the ultra-high, high, and low concentrations (500,000, 8000, and 300 ng/mL). The QCs demonstrated room temperature stability up to 22 hours and up to 5 freeze-thaws with %RE within ± 25%. The stability QCs over recovered but were less than 25% RE (**Figure 7**).
Furthermore, samples were prepared under different interfering conditions to evaluate the selectivity and specificity of the VAMS collection method which demonstrated a proper recovery of the analyte. Selectivity and specificity acceptance criteria were met at 100% of the tested samples at the high QC (8000 ng/mL) and LLOQ (100 ng/mL) levels (**Figure 8**).



Figure 3. Whole blood extracted samples that have been prepared with human plasma (left) and human serum (right) after centrifugation.

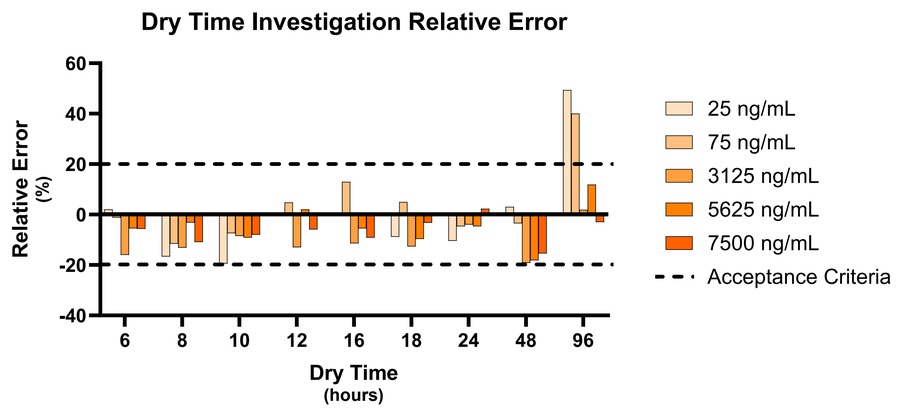
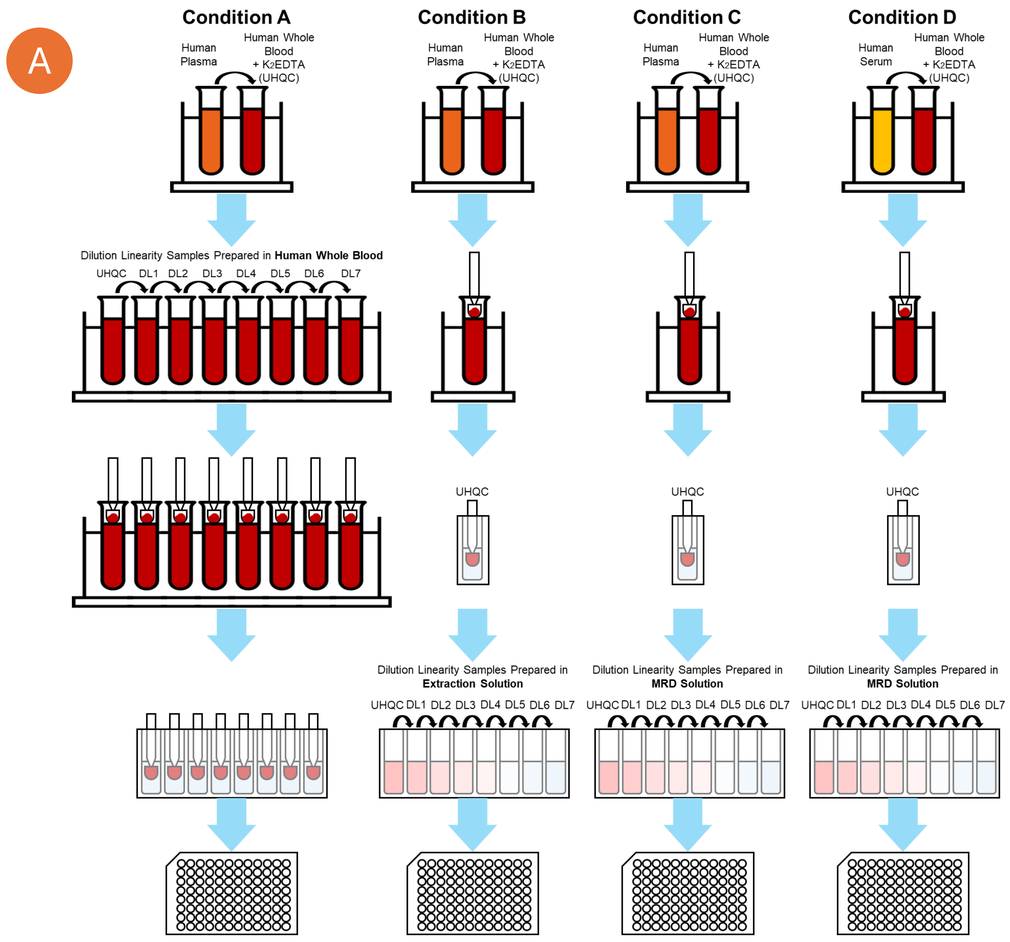


Figure 4. Relative error of the VAMS control samples (25, 75, 3125, 5625, and 7500 ng/mL of the anti-malarial monoclonal antibody drug candidate) when dried at 6, 8, 10, 12, 16, 18, 24, 48 and 96 hours with acceptance criteria at ± 20% RE.



B

Nominal Concentration (ng/mL)	Condition A	Condition B	Condition C	Condition D
UHQC	500,000	500,000	500,000	500,000
DL1	25,000	100,000	100,000	50,000
DL2	5,000	10,000	10,000	5,000
DL3	1,000	1,000	1,000	1,000
DL4	200	200	200	200
DL5	40	40	40	40
DL6	8	8	8	8
DL7	1.6	1.6	1.6	N/A

Figure 5. (A) Scheme of dilution linearity experiments tested with UHQC at 500,000 ng/mL. **(B)** Overview of the dilution linearity experiments using a calibration curve range of 12.5-3125 ng/mL with the nominal concentrations of the samples for conditions A-D. Samples in the range of quantitation (shaded in orange) were evaluated for recovery with an acceptance criterion of ± 25% RE, where samples within ± 25% RE of the nominal concentration are in green, and samples outside ± 25% RE are in red.

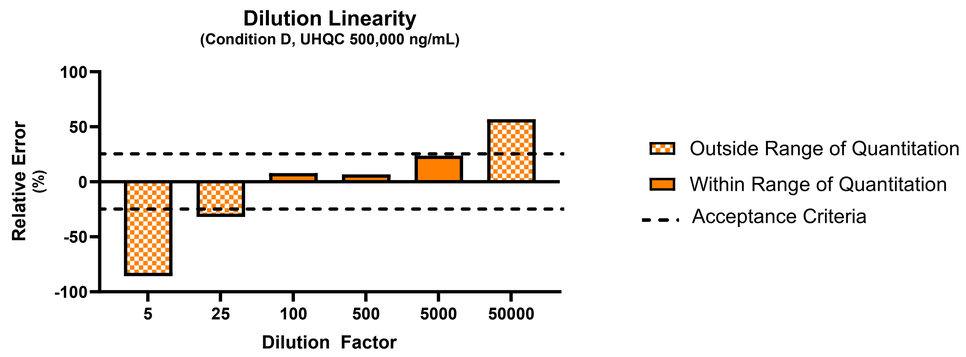


Figure 6. Dilution linearity results using condition D with a calibration curve range of 100-10,000 ng/mL and an acceptance criteria of ± 25% RE.

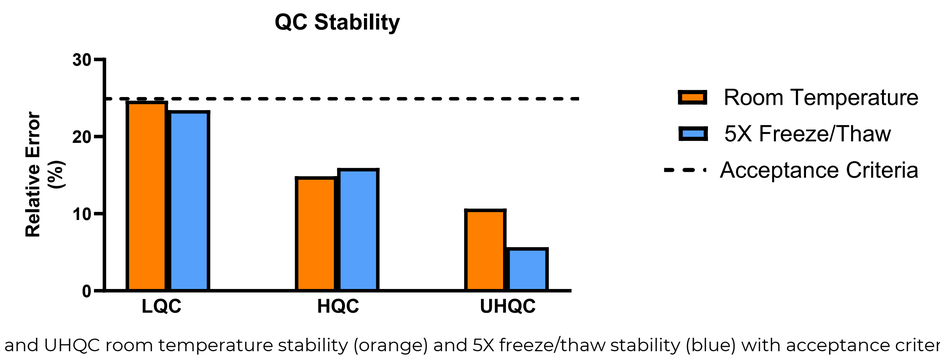


Figure 7. LQC, HQC, and UHQC room temperature stability (orange) and 5X freeze/thaw stability (blue) with acceptance criteria at 25% RE.

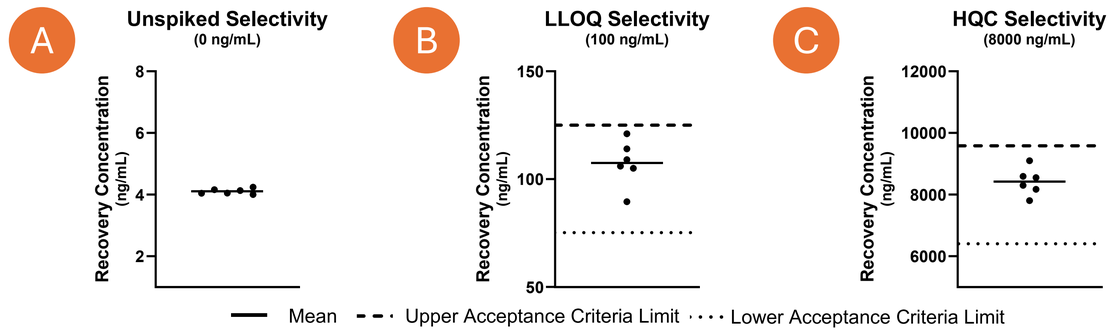


Figure 8. (A) Unspiked selectivity sample concentration recovered. All samples recovered below the LLOQ (100 ng/mL). **(B)** LLOQ selectivity sample concentration found with acceptance criteria ± 25% RE of 100 ng/mL. **(C)** HQC selectivity sample concentration found with acceptance criteria ± 20% RE of 8000 ng/mL.

CONCLUSION
The results of this investigation study demonstrated the ability to adapt the VAMS devices in our laboratory processes which will significantly reduce the need for large blood volumes, mitigate the risks associated with invasive sampling, and streamline the bioanalysis.
This work lays the foundation for the use of VAMS in quantitative bioanalysis, offering a reliable and efficient alternative for small volume human whole blood collection over the traditional large volume collection of matrices in clinical studies.