Population Research Studies: Evaluation of the Health Impact from Environmental Exposures among Military Personnel

Exposure Screening and Biorepository Opportunities for Capillary Blood Draws – Test Case for PFAS analysis using the OneDraw Capillary Blood Sampling Unit

Andrew J. Morris[1], Lauren A. Havens[1], Elizabeth R. Heitz[2], Kelsie R. Hartley[1], Jiajun Lei[3], Kruttika Dabke[3], Jonathan E. Katz[3,4,5], Spyridoula Maraka[1],Warren Casey[6], Jerry S.H. Lee[3,4,7], Craig D. Shriver[8], Terra D. Vincent-Hall[2]

[1]Central Arkansas Veterans Affairs Healthcare System, Little Rock, AR; [2]Exposure Science Program, Health Outcomes Military Exposures, Department of Veterans Affairs, Washington, DC; [3]Ellison Medical Institute, Los Angeles, CA; [4]Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; [5]Murtha Cancer Center Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD. [6]Division of Translational Toxicology, National Institute of Environmental Health Sciences, [7]Department of Chemical Engineering and Material Sciences, Viterbi School of Engineering, University of Southern California, Los Angeles, California, [8]Murtha Cancer Center Research Program, Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD

Correspondence: AJMorris@uams.edu

Introduction

Military personnel and veterans are frequently exposed to occupational and environmental hazards, including persistent chemicals, such as PFAS. Traditional biomonitoring methods rely on venous blood draws, necessitating clinical infrastructure and restricting accessibility in field settings. This study evaluated the feasibility of dried capillary blood sampling onto paper for exposure assessment (**Figure 1**).

We selected the OneDraw device for dried blood collection due to its robustness, ease of use, and minimal post-collection handling.

Our evaluation focused on the feasibility, reproducibility, and analytical sensitivity of dried blood collection compared to traditional venous blood-based measures for PFAS screening. Additionally, to explore broader utility in exposure monitoring, we conducted untargeted metabolomics to assess other environmental chemicals present in the samples.

Materials & Methods

Veteran patients were recruited from the Central Arkansas Veterans Healthcare System. Each participant (N=133) responded to a brief questionnaire intended to capture potential sources of PFAS exposure (e.g., drinking water source, occupation) and then had time-matched blood drawn both by venipuncture into a 4 mL "purple top" EDTA tube and capillary blood from the upper arm using the OneDraw device. The self-contained OneDraw device acquires two 75-uL samples of capillary blood onto filter paper, which were subsequently dried in the separately provided sample carrier. PFAS extraction was performed using an optimized extraction and a UHPLC-coupled tandem, triple quadrupole mass spectrometry workflow (Sciex 7500 Q-Trap) with data processing and reporting done using Sciex OS Multiquant software. Statistical methods were used to assess relationships between PFAS levels in dried blood (DBS) and plasma. Plasma PFAS concentrations were also modeled in relation to self-reported environmental exposure factors, including proximity to known PFAS contamination sources (e.g., military installations, industrial sites) and primary drinking water sources, adjusting for military and civilian occupational exposures and key demographic factors.

A subset of individuals (N=30), comprising 15 participants each from the highest and lowest quartiles of total PFAS levels, was selected for untargeted metabolomics analysis. Briefly, each dried blood strip was bisected lengthwise: one half was stored for future use, while the other underwent organic extraction and was analyzed using four LC-MS runs, incorporating both HILIC and C18 chromatography under positive and negative ionization modes.

Results

Limits of quantitation in DBS were 0.1 ng/ml for most PFAS, which is comparable to widely reported assay performance for these measurements in plasma.

We compared the matched venous blood plasma measures to those we obtained from the DBS samples. The regression analysis indicated that DBS levels of PFAS were strongly predictive of levels in plasma, while the correlation analysis revealed significant, strong, positive correlations between DBS and plasma PFAS levels.

To assess the potential of dried blood spot analysis beyond PFAS compounds for exposure monitoring, we screened the 30 samples selected for untargeted metabolomics for ion signals consistent with 20 exogenous compounds commonly known to present in various populations (**Figure 6**). Of these, compounds with additional validation (data not shown) are labeled by name. Finally, we observed concordance between our validated PFAS assay for PFHxS and an m/z based presumptive PFHxS signal identified in the untargeted dataset, suggesting that routine "digitization of biology" during biobanking could facilitate rapid hypothesis testing for emerging exposure compounds.

Conclusions

In this study we demonstrated the **feasibility of capillary blood sampling for PFAS level assessment.** Dried capillary blood proved to be a viable alternative to traditional venous blood draws for PFAS exposure assessment, offering advantages in accessibility and ease of use in field settings. Further, in our comparison **we show that capillary blood has a strong correlation with plasma levels.** In general, PFAS concentrations measured in dried blood spots (DBS) showed strong correlation with matched plasma samples.

Finally, we **demonstrate potential for broad compound surveillance** by showing not only the PFAS measures but sensitivity for a broad range of compounds. Beyond immediate screening, these workflows also enable facile long-term biobanking for "just-in-time" acquired samples. Both screening and biobanking can be performed with a considerably **reduced dependence on clinical infrastructure.**

Conflict of Interest

The authors declare no competing financial interest.

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Experimental Flow Diagram

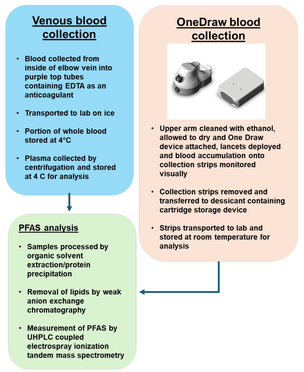

Figure 1. Workflow comparing traditional venous blood collection with capillary blood collection using the OneDraw device for per- and polyfluoroalkyl substances (PFAS) exposure assessment.

Venous blood was collected into EDTA tubes, processed via centrifugation, and plasma stored at 4°C. OneDraw blood sampling involved automated collection from the upper arm onto dried blood strips, stored at room temperature. Both sample types underwent PFAS extraction and quantification using UHPLC–tandem mass spectrometry, following organic solvent extraction, protein precipitation, and lipid removal. This workflow supports comparison of sample types for scalable biomonitoring.

Sample Population

Characteristics of Veterans sampled for PFAS blood concentrations using OneDraw device

Characteristic	Participants (N=133)
Demographics	
Age, years at blood draw	
Range	34 - 80
Mean (SD)	61.4 (11.9)
Sex	
Male	71.4% (95)
Female	28.6% (38)
Race and ethnicity	
White, non-Hispanic	63.2% (84)
Black or African American, non-Hispanic	36.1% (48)
White, Hispanic	0.8% (1)
County of residence	
Faulkner	9.8% (13)
Lonoke	12.0% (16)
Pulaski	54.9% (73)
Saline	15.0% (20)
Other, including out of state	8.3% (11)
Self-reported exposure factors	
Ever used AFFF for firefighting while in the military	
Yes	17.3% (23)
No, but involved in firefighting	7.5% (10)
No, never involved in firefighting	75.2% (100)
Primary source of drinking water	
Municipal	54.1% (72)
Bottled	39.9% (53)
Private well	3.0% (4)
Do not know or not reported	3.0% (4)
Exposure to stain repellent or water protective coatings	
Yes	46.6% (62)
No	44.4% (59)
Do not know	9.0% (12)
Donated blood in the past 12 months	
Yes	6.0% (8)
No	94.0% (125)

Table 1. Participant demographics

Subjects were consented and samples drawn from patients receiving care at the John L. McClellan Memorial Veterans' Hospital in Little Rock, Arkansas between Dec-2023 and Oct-2024. Summary characteristics are presented in this table based on replies to the brief questionnaire administered during consent.

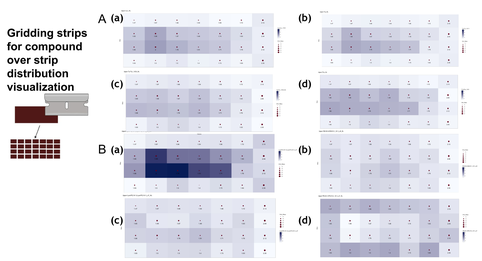

Figure 2: Heatmap view of Amino Acids and Phospholipid retention profile over the OneDraw strips. Panel **A** includes the following amino acids (a)Leu, (b)Trp, (c)Tyr, (d)Phe, and panel **B** include the following phospholipids: (a)LysoPC(18:1), (b)SM(36:2), (c)LysoPE(18:1), and (d)PE(34:1). The metabolite/IS ratios across each strip are normalized to the grid with the lowest ratio, and the heatmap color upper range uses the highest metabolite/IS ratio across all metabolites included. The grid masses are marked at the center of each grid. (Figure adapted from Lei, Katz, Proceedings of the American Society of Mass Spectrometry, 2024)

PFAS Quantitation Method Validation

Analyte Abbreviation	Plasma LOQ (ng/mL)	DBS LOQ (ng/mL)	Plasma Frequency (%)	DBS Frequency (%)	Plasma Range (ng/mL)	DBS Range (ng/mL)
PFBS	0.1	0.1	0.00	0.00	<LOQ	<LOQ
PFPES	N/A Plasma	0.1	N/A	0.00	N/A	<LOQ
PFHxS L	0.08	0.08	97.74	89.47	<LOQ-3.40	<LOQ-1.86
PFHxS BR	0.1	0.1	58.65	24.06	<LOQ-0.91	<LOQ-0.43
PFHxS Total	0.1	0.1	99.25	91.73	<LOQ-3.96	<LOQ-2.24
PFHpS	0.1	0.1	54.89	15.04	<LOQ-0.56	<LOQ-0.32
PFOS L	0.08	0.08	100.00	98.50	0.08-16.83	<LOQ-5.91
PFOS Br	0.1	0.1	98.50	93.23	<LOQ-11.78	<LOQ-5.26
PFOS Total	0.1	0.1	100.00	98.50	0.16-22.76	<LOQ-8.81
PFNS	0.1	0.1	0.75	0.00	<LOQ-0.59	<LOQ
PFDS	0.1	N/A DBS	0.00	N/A	<LOQ	N/A
PFBA	0.3	0.3	NR	NR	NR	NR
PFPeA	0.1	0.1	NR	NR	NR	NR
PFHxA	0.1	0.3	0.00	0.00	<LOQ	<LOQ
PFHpA	0.1	0.1	0.75	0.00	<LOQ-0.13	<LOQ
PFOA	0.1	0.1	98.50	92.48	<LOQ-2.64	<LOQ-1.03
PFNA	0.1	0.1	90.98	42.86	<LOQ	<LOQ-0.36
PFDA	0.1	0.1	43.61	15.79	<LOQ-1.47	<LOQ-0.71
PFUdA	0.1	0.1	10.53	7.52	<LOQ-0.45	<LOQ-0.19
PFDoA	0.1	0.1	1.50	0.00	<LOQ-0.19	<LOQ
PFTrDA	0.1	N/A DBS	0.00	N/A	<LOQ	N/A
PFTeDA	0.1	0.3	0.00	0.00	<LOQ	<LOQ
HFPO-DA	0.1	0.1	0.00	2.26	<LOQ	<LOQ-0.12
NaDONA	0.3	0.3	0.00	0.00	<LOQ	<LOQ
FOSA	0.3	0.3	0.00	14.29	<LOQ	<LOQ-3.67
N-MeFOSAA L	0.08	0.08	15.79	6.02	<LOQ-1.86	<LOQ-0.91
N-MeFOSAA BR	0.1	0.1	3.76	0.75	<LOQ-0.58	<LOQ-0.42
N-MeFOSAA Total	0.1	0.1	21.05	6.02	<LOQ-2.44	<LOQ-1.33
N-EtFOSAA L	0.08	0.08	0.00	0.00	<LOQ	<LOQ
N-EtFOSAA Br	0.1	0.1	0.00	0.00	<LOQ	<LOQ
N-EtFOSAA Total	0.1	0.1	0.00	0.00	<LOQ	<LOQ
4:2 FTS	0.1	0.3	0.00	0.00	<LOQ	<LOQ
6:2 FTS	10	10	0.00	0.00	<LOQ	<LOQ
8:2 FTS	0.1	0.1	0.00	0.00	<LOQ	<LOQ
9Cl-PF3ONS	25	0.1	0.00	0.00	<LOQ	<LOQ
FBSA	10	0.3	0.00	0.00	<LOQ	<LOQ
FHxSA	N/A Plasma	20	N/A	0.00	N/A	<LOQ

Table 2. PFAS method validation and detection limits for 30 PFAS analytes

Assay validation was performed across five batches to assess inter- and intraday performance. Calibration curves met accuracy criteria (≤20% at the lowest point, ≤15% otherwise) with $R^2 > 0.99$. The limit of detection (LOD) was 0.1 ng/mL (S:N > 3), and the limit of quantitation (LOQ) was equivalent to the LOD (S:N > 10) for 21 analytes. Most analytes (21/30) were quantifiable over 0.1–25 ng/mL; three additional analytes had a range of 0.3–25 ng/mL. Some compounds showed reduced recovery (~50%) in plasma or dried blood. Analytes are color-coded by coefficient of variation (CV): green (<20%), orange (<30%), and red (>30%). Results confirm PFAS can be reliably quantified in dried blood, with comparable performance to plasma, and no contamination from OneDraw strips. Linear and total values are reported separately where isomers were chromatographically resolved.

PFAS Measurement Concordance: Venous vs Capillary

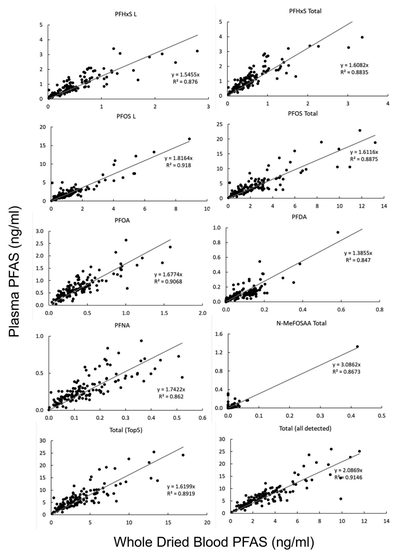

Figure 3: Correlation of clinical PFAS measurements against our DBS measurements. To assess the relationship between PFAS levels in plasma and OneDraw-collected whole blood, we generated scatter plots for the most frequently detected compounds and conducted linear regression analyses. Strong correlations were observed, with R^2 values exceeding 0.9 for PFOS, PFHxS, PFOA, and N-MeFOSAA, and above 0.85 for PFDA and PFNA. The combined regression for the five most commonly detected PFAS yielded an R^2 of 0.93, while the correlation across all detected PFAS was 0.90. **These findings indicate that PFAS concentrations in venous plasma are highly predictive of those in capillary whole blood.** Notably, the regression slopes were consistently less than one, reflecting the expected lower PFAS concentrations in whole blood compared to plasma.

Analytes	Pearson's Correlation	Pearson's Correlation P	Spearman's Rank Correlation	Spearman's Rank Correlation P
PFHxS	0.92	>0.001	0.93	>0.001
PFHpS	0.81	>0.001	0.86	>0.001
PFOS	0.92	>0.001	0.88	>0.001
PFOA	0.93	>0.001	0.92	>0.001
PFNA	0.85	>0.001	0.87	>0.001
PFDA	0.94	>0.001	0.83	>0.001
PFUdA	0.86	>0.001	0.77	>0.001
N-MeFOSAA	0.88	>0.001	0.62	>0.001

Table 3. Correlations between OneDraw whole dried blood and venous blood plasma PFAS levels.

To evaluate the relationship between PFAS concentrations in plasma and OneDraw whole blood, we calculated both Pearson's and Spearman's rank correlation coefficients. Strong positive correlations (r > 0.7) were observed for 8 PFAS compounds, including PFOA, PFHxS, PFHpS, PFOS, PFDA and N-MeFOSAA. Moderate correlations (r between 0.4 and 0.7) were also observed for several other compounds (data not shown). All reported correlations were statistically significant, indicating a consistent and meaningful relationship between PFAS levels in plasma and those measured in capillary whole blood collected with the OneDraw device.

Untargeted Metabolomics

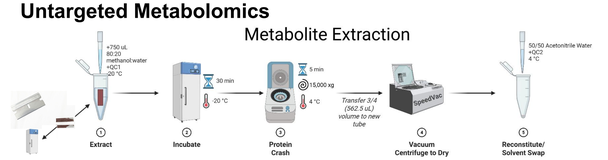

Figure 4. Processing and biobanking for untargeted metabolomics

OneDraw collects samples onto 2 paper strips, one of which was used for the PFAS analysis shown above. In order to pilot the utility of an untargeted workflow for biobanking and exposure screening, 30 of the secondary strips (15 each of those identified in the top quartile and bottom quartile for PFAS levels) were processed and run as shown above. Briefly, samples were extracted with organic solvent, dried and resuspended for analysis either in C18 or HILIC chromatography coupled to a Bruker TIMS TOF 2. Data were collected in positive and negative ionization modes in separate runs.

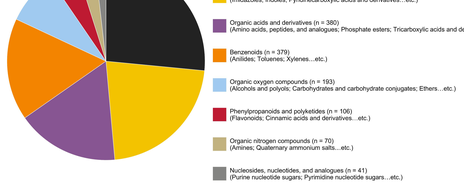

Figure 5. Feature identification and class assignment

Features were matched against mass based spectral libraries for the purposes of preliminary identification. Features from the 4 runs described in Figure 4 were deduplicated and assigned to compound classes.The table and pie chart show the counts of compounds in the various "Superclasses" based on those used by ClassyFire (*Djoumbou Feunang Y, Eisner R, Knox C, Chepelev L, Hastings J, Owen G, Fahy E, Steinbeck C, Subramanian S, Bolton E, Greiner R, and Wishart DS: ClassyFire: Automated Chemical Classification With A Comprehensive, Computable Taxonomy. Journal of Cheminformatics, 2016, 8:61.*).

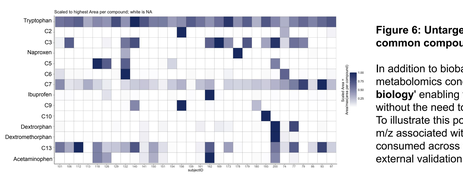

Figure 6: Untargeted "Exposure Screening" for known common compounds of exposure.

In addition to biobanking every sample, performing untargeted metabolomics concurrently offers the opportunity to '**digitize biology**' enabling first-pass screening for emerging compounds without the need to retrieve samples from the biobank. To illustrate this potential, we screened our samples for the target m/z associated with several compounds known to be widely consumed across the population. Compounds for which we had external validation are named in the table.

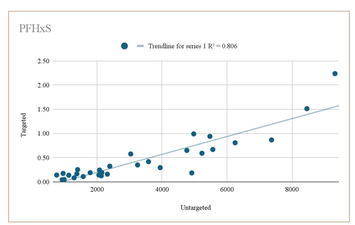

Figure 7: Comparison of our Untargeted analysis of PFHxS against our validated assay.

In this poster, we present a validated method for the quantitative analysis of 27 PFAS compounds using commercially available standards. This required targeted mass spectrometry—focusing exclusively on those compounds—to achieve optimal sensitivity and specificity.

To explore the potential of untargeted metabolomics for screening emerging compounds, including those without available standards, we searched our untargeted data for four of these PFAS compounds - shown is PFHxS. While subject to certain limitations, this approach illustrates the potential to interrogate novel compounds in silico, without the need for physical reanalysis. Similar results were obtained for PFOS. Our untargeted method did not detect PFNA nor PFOA.

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