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

Whole blood microsampling for environmental exposure biomonitoring.

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Introduction

Exposure to environmental chemicals is an established modifier of communicable disease risk and of particular concern to service members and veterans who often work with toxic environmental chemicals or live near sites of environmental contamination. Traditional exposure monitoring requires access to both clinical infrastructure and temperature-controlled sample handling. Accessible monitoring of exposure to these chemicals in complex environments (such as forward deployments) requires facile approaches to collect and stabilize sample material for subsequent analysis. Whole blood microsampling, for example dried blood spots (DBS), holds promise for this purpose.

We have evaluated devices that enable self-administered collection, stabilization, and storage of whole blood onto paper strips (DrawBridge Health OneDraw) or volumetric absorbent materials (Tasso Tile T20) for monitoring exposure to different classes of environmental chemicals that include per- and polyfluoroalkyl substances (PFAS) and a panel of multiple classes of hydrophobic semi-volatile environmental chemicals. These devices enable collection of two (OneDraw) or 4 (Tasso) separate volumetric dried blood samples that can then be stored for relatively long periods of time and then analyzed using different sample preparation and analytical workflows to measure environmental chemicals using GC and LC coupled multistage/high resolution mass spectrometry. Complementing our previous work, we show here the Tasso T20 is similarly suitable for PFAS exposure biomonitoring with limits of quantification that are higher than with OneDraw because of the smaller volumes of whole blood that are collected (0.5 vs 0.1 ng/ml). Additionally, we expand our exposure validation and demonstrate that the Tasso T20 is also highly effective for measurement of other classes of environmental chemicals including PCBs, PCDEs, and pesticides at levels commonly observed in the general US population using gas chromatography electron impact ionization high resolution mass spectrometry.

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, see **Table 1**) and then had time-matched blood drawn both by venipuncture and using the OneDraw device (as in **Figure 1**). PFAS were measured after extraction and removal of lipids using a UHPLC-coupled tandem, triple quadrupole mass spectrometry workflow (Sciex 7500 Q-Trap). Statistical methods were used to assess relationships between PFAS levels in 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 and analyzed with the FluoroMatch software.

Validation of Tasso for PFAS measurements was done using sheep blood fortified with PFAS and NIST 1957 serum with quantification of PFAS by LC-MS as described above. Validation of Tasso for measurement of a panel of environmental chemicals was done using NIST 1958 serum. Sample preparation involved biphasic organic extraction and MgSO4 dehydration and direct analysis of the remaining material to minimize analyte losses during evaporation/ reconstitution.

Results

DBS PFAS levels strongly predicted and correlated with plasma levels (**Figure 2**). Location data from 72.9% of participants were mapped against potential Arkansas exposure sources, including oil and gas wells, manufacturers, Clinton National Airport, and military installations (**Figure 3**).

Accuracy and reproducibility of measurements for most PFAS was acceptable using both Tasso and OneDraw (**Table 2**). Measurements of PFOS were not accurate using Tasso because of a sorbent background signal corresponding to approximately 9 ng/mL in the starting sample (**Figure 4**).

Untargeted PFAS screening with FluoroMatch identified candidate compounds and relative abundance patterns (**Figure 5**). Broader metabolomic screening also detected signals consistent with 20 common exogenous compounds (**Figure 6**).

Conclusions

This study demonstrates that dried capillary blood is a practical alternative to venous blood sampling for PFAS assessment, with dried capillary blood concentrations strongly correlating with plasma levels. The approach also supports broad compound surveillance and long-term biobanking while reducing reliance on clinical infrastructure. A potential workflow of the future would involve just-in-time biosampling of dried blood with a bipartite workflow of analysis for real-time biosurveillance and biorepository storage to enable future analyses (**Figure 7**).

Conflict of Interest

The authors declare no competing financial interest.

Acknowledgements

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MHS Military Health System
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Experimental Flow Diagram

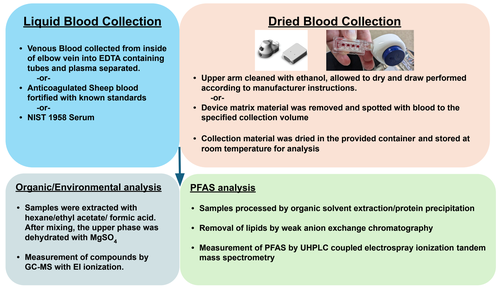

Figure 1. Workflow comparing compound analysis derived from whole and dried blood samples.

Samples consisting of either liquid or dried blood and were either purchased as standards (sheep's blood or NIST 1958) or acquired via phlebotomy. The samples were then either submitted for certified PFAS analysis (Eurofins), processed in-house for PFAS analysis via LCMS, or processed in-house to assess organic and environmental compounds via GCMS.

Sample Population - Section 1

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.

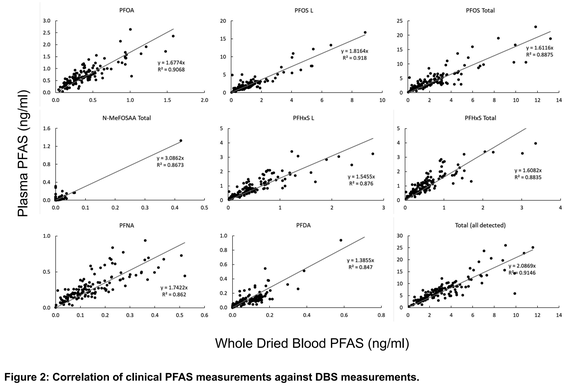

Figure 2: Correlation of clinical PFAS measurements against DBS measurements.

PFAS levels in plasma and OneDraw-collected whole blood were measured and scatter plots for the most frequently detected compounds are presented along with 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. **These findings indicate that PFAS concentrations in venous plasma are highly predictive of those in capillary whole blood.** We note, the regression slopes were consistently less than one, reflecting the expected lower PFAS concentrations in whole blood compared to plasma.

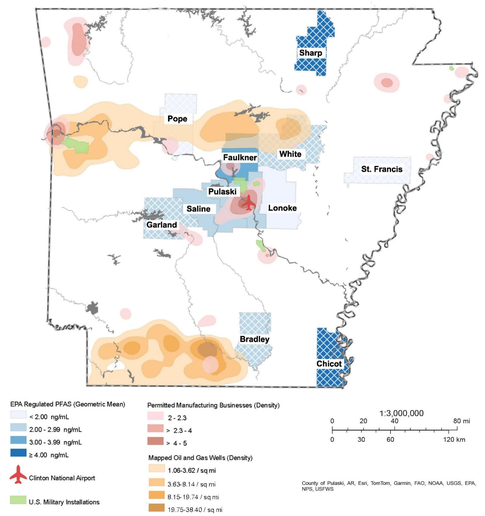

Figure 3. County-level distribution of total target PFAS concentrations and potential environmental sources among Arkansas Veterans

County-level distribution of total target PFAS concentrations in dried whole capillary blood or plasma among 97 Veterans (72.9% of the study population) who reported residing in the same Arkansas county during the previous five years. Participants represented 11 counties; among counties with at least five participants, geometric mean concentrations were highest in Faulkner County (3.13 ng/mL; 95% CI, 2.06–4.76), followed by Pulaski, Saline, and Lonoke Counties, although county-level differences were not statistically significant (p=0.1649). The maps also display potential environmental PFAS sources, including areas of elevated manufacturing density, oil and gas well density, Clinton National Airport, and military installations; counties without eligible Veteran participants were not shown.

	Tasso		OneDraw	
Component Name	1.5 ug/mL	10 ug/mL	1 ug/mL	10 ug/mL
PFBS	129.6	117.9	94.0	88.7
PFPeS	112.2	95.3	86.9	81.8
PFHxS L	144.2	110.9	90.5	84.1
PFHxS Total	118.8	107.8	87.7	79.8
PFHpS	166.6	114.4	84.4	85.5
PFOS L	706.4	213.0	87.5	83.6
PFOS Total	758.8	186.1	87.8	82.8
PFNS	137.0	104.1	88.0	81.8
PFDS	88.1	74.8	65.1	61.5
PFBA	128.0	116.2	93.0	85.5
PFPeA	106.4	125.6	91.6	85.2
PFHxA	127.7	127.9	91.3	83.3
PFHpA	129.6	116.7	92.7	84.2
PFOA	115.8	125.3	93.6	82.1
PFNA	127.4	126.1	95.7	83.2
PFDA	128.7	112.8	95.0	88.5
PFUdA	150.7	122.4	96.9	88.4
PFDoA	127.9	110.6	99.0	92.1
PFTrDA	165.4	158.8	153.3	166.4
PFTeDA	115.2	113.9	92.4	80.8
HFPO-DA	94.8	104.9	85.7	81.2
NaDONA	76.7	97.9	102.0	86.5
11Cl-PF3OUdS	218.6	192.6	68.9	61.0
FOSA	120.2	119.6	92.4	83.3
N-MeFOSAA L	116.1	124.0	91.0	85.0
N-MeFOSAA Total	125.7	113.7	93.9	82.6
N-EtFOSAA L	138.8	111.0	86.2	83.2
N-EtFOSAA Total	175.2	112.5	87.4	86.0
4:2 FTS	143.2	122.8	83.0	68.1
6:2 FTS	113.4	130.0	87.2	73.2
8:2 FTS	124.1	113.6	90.9	76.2
9Cl-PF3ONS	217.3	227.6	99.3	92.6
FBSA	40.5	36.8	64.7	49.7
FHxSA	66.1	52.5	157.4	113.5

Table 2. Accuracy and consistency of PFAS measurements in whole blood using OneDraw or Tasso.

Sheep whole blood was spiked with a mixture of PFAS at the indicated concentration (1-10 ug/mL) and absorbed onto the indicated matrix, either TASSO or OneDraw. After the samples were allowed to dry, PFAS analysis was performed, and the percent CV for triplicate analyses is shown. Green indicates CVs < 20%, Yellow < 40% and Red > 40%. We note that OneDraw has consistently lower CVs than Tasso which can be explained by the smaller volume of blood absorbed onto a Tasso tile compared to a OneDraw paper strip. Measurements of PFOS alone are highly inaccurate using Tasso because of a high baseline signal derived from the matrix itself (see Figure 4 below).

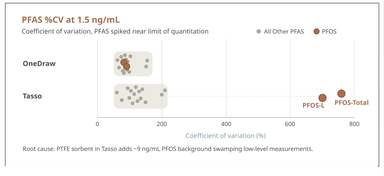

Figure 4. Accuracy of PFAS measurements in dried blood using OneDraw or Tasso.

For each device, a point cloud is shown representing all the PFAS compounds tested. Specifically indicated are the PFOS species. As shown above, at the lower concentrations of analysis, the PFOS signal in Tasso has an exceptionally high CV resulting from our estimated background signal deriving from the device itself corresponding to ~ 9 ng/mL in the starting blood sample.

Untargeted PFAS/Exposure Interrogation

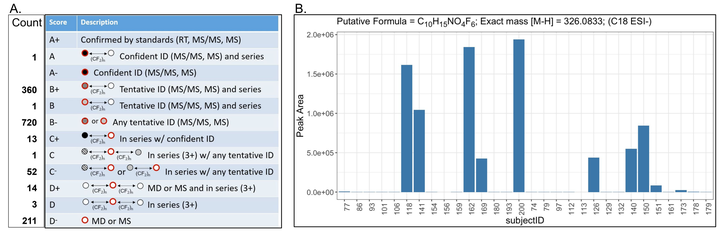

Figure 5. Untargeted identification of potential exposure compounds of interest - PFAS workflow

To evaluate the utility of an untargeted workflow for identifying novel PFAS compounds, we selected 30 participants from the original cohort from Table 1. Participants were drawn from the upper (N=15) and lower quartiles (N=15) of total PFAS exposure. Samples were analyzed by untargeted LC–MS using a C18 column in negative-ion mode with both full scan MS^1 and data dependent acquisition of MS^2 spectra. The resulting data were processed with FluoroMatch which assesses molecular features and fragments to identify putative PFAS compounds. Panel **A** summarizes the number of compounds assigned to each FluoroMatch identification-confidence level. Panel **B** shows the ion intensities of a selected feature of interest across the 30 participants. Although the proposed molecular formula was not confirmed, the feature was detected in only ~one-third of participants suggesting an externally derived compound rather than being of endogenous biological origin.

Expanded Compound Analysis - Validation in NIST 1958

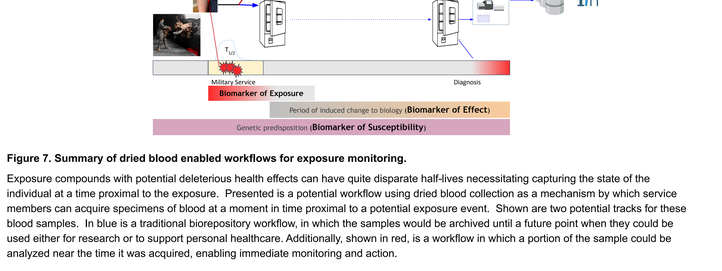

Figure 6. Use of Tasso for measurement of an expanded range of environmental chemicals by GC HR mass spectrometry.

A: We established a GC high resolution mass spectrometry method for measurement of 360 environmental chemicals in multiple classes. In brief this uses a Thermo Orbitrap Exploris 240 instrument system with data collected in electron impact ionization mode. We made a compound database using observed or reported information for environmental chemicals of interest. Peak finding, spectral deconvolution, compound identification and quantification were done with Thermo Tracefinder. The method uses compound class specific mass labeled internal standards (anthracene, mirex, DDE, permethrin, PCBs 81, 101, 153 and 180). The sample preparation is designed to minimize dilution and avoid solvent evaporation by combining organic solvent extraction/deproteination with sample dehydration. Mean recoveries of the internal standards spiked into NIST 1958 serum either alone or after absorption onto Tasso were 50% and 40% respectively. Calibration of measurements of PCBs 153, 101 and 180 using their corresponding internal standards showed excellent linearity. Measurements of PCBs 153, 101 and 180 in NIST 1958 serum with or without prior absorption onto Tasso were within 20% of the true values. Overall this shows that absorption of NIST 1958 serum onto Tasso does not impair measurements of environmental chemicals using our GC HRMS workflow.

B: To investigate the ability of our method to detect a broader range of environmental chemicals, NIST SRM 1958 serum was again extracted before and after spotting onto TASSO sorbent and dried. After drying, the material was extracted with hexane, ethyl acetate, and formic acid using vigorous shaking. Following centrifugation, the upper phase was removed, dehydrated with magnesium sulfate, and analyzed using our GC HRMS method. The resulting GC-MS data were processed using Thermo Tracefinder and searched against our environmental chemical database, ~100 compounds were identified of which 47 had acceptable ratios of target and qualifier ions and retention time matches to authentic standards. After normalization to our internal standards and correction for differences in volume, a strong correlation was observed between peak areas for these 47 compounds in NIST serum versus NIST serum absorbed to Tasso. In addition to a series of PFAS, additional chemicals detected in both samples included a series of organochlorine compounds used as pesticides, herbicides and fungicides. These findings suggest that the TASSO Tile T20 is highly suitable for monitoring exposure to a broader range of environmental chemicals.

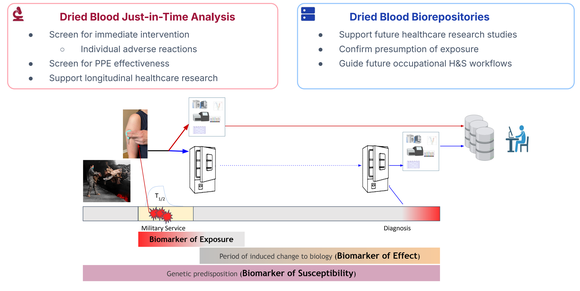

Figure 7. Summary of dried blood enabled workflows for exposure monitoring.

Exposure compounds with potential deleterious health effects can have quite disparate half-lives necessitating capturing the state of the individual at a time proximal to the exposure. Presented is a potential workflow using dried blood collection as a mechanism by which service members can acquire specimens of blood at a moment in time proximal to a potential exposure event. Shown are two potential tracks for these blood samples. In blue is a traditional biorepository workflow, in which the samples would be archived until a future point when they could be used either for research or to support personal healthcare. Additionally, shown in red, is a workflow in which a portion of the sample could be analyzed near the time it was acquired, enabling immediate monitoring and action.

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