•Dried blood Spot • Remote Sampling • Digitizing Biology

OPPORTUNITIES FOR REMOTE SAMPLING TECHNOLOGIES TO ENABLE COMPREHENSIVE BIOBANKING OF MILITARY POPULATION TOWARDS DIGITIZING BIOLOGY

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BACKGROUND

Strategies toward comprehensive biobanking of biological samples have immense scientific and clinical value understanding the impact of military exposures, as creating “snapshots” of an individual’s current biological state that can be later interrogated for questions of both health and exposure status. Current sampling mechanisms include regular intravenous blood draw in between deployments, as well as wearable sensors. Intravenous blood sampling, however, is not accessible in many field scenarios, as its invasive nature limits the practical sampling frequency, and it is usually accompanied with stringent sample storage condition requirements. Additionally, wearable devices are not effective for monitoring unknown hazards nor the precise and comprehensive individual exposure data that can be obtained from in-vivo measurements. Consequently, alternative sampling approaches that enable frequent blood sampling would be ideal towards screening and comprehensive biobanking. The development of capillary blood microsampling kits are advantageous for frequent sampling purposes for their minimal invasiveness and much less stringent storage condition requirements. In this project, the suitability of various remote sampling kits will be systematically assessed to identify promising candidates for real-world application.

HIGHLIGHTS

- **Dried blood spot technologies (DBS) produce mostly comparable data.**
- **The OneDraw device requires the least training for the most reproducible sample acquisition.**
- **The OneDraw device does show some chromatography effects that need to be considered if partial strip processing is desired. DBS spots on “Guthrie” style cards do not show this effect.**

ANALYTICAL METHODS (LC-MS)

Hydrophilic Interaction LIquid Chromatography (HILIC) Method:
Flow Rate: 400 µL/min, Column Temp: 10 ℃, Injection Volume: 2 µL

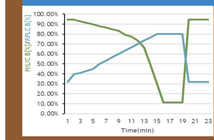
Mobile Phase:
A: 90:10: H2O+20mM CH_3COONH_4+5µM Medronic Acid (PH 9.4):ACN
B: 90:10: ACN:H2O+20mM CH_3COONH_4+5µM Medronic Acid (PH 9.4)

Column: InfinityLab Poroshell 120 HILIC-Z 100 x 2.1 mm, 2.7 µm

Reverse Phase Liquid Chromatography Method:
Flow Rate: 500 µL/min, Column Temp: 50 ℃, Injection Volume: 2 µL

Mobile Phase:
A: 60:40: ACN: H2O + 10mM $HCOONH_4$
B: 90:8:2 IPA:ACN:H2O + 10mM $HCOONH_4$

Column: ZORBAX Eclipse Plus C18 50 x 2.1 mm, 1.8 µm



Mass Spectrometry (MS) Method
Instrument: Bruker TIMSTOF Pro 2

Capillary Voltage: 4500 V (POS/NEG)
Nebulizer Gas: 2.0 Bar
Sheath Gas: 275 ℃, 4.0 L/min
Dry Gas: 230 ℃, 8.0 L/min
Transfer Time: 54.0/65.0 µs for NEG/POS mode
Scan Mode: MS (50 - 1250 m/z) @ 4.00 Hz

EVALUATION STRATEGY

For sampler evaluation, blood was obtained from a single individual in replicate using the various sampler modalities within a one hour period. Samples were extracted with 80/20 methanol/water, dried, and resuspended for LC/MS analysis. A separate set of samples was taken to assess the across strip and intraspot variability in the OneDraw and paper card modalities respectively. Each sample was measured in four LC/MS runs which coupled either HILIC or C18 chromatography to an electrospray source operated in either positive or negative mode.

For intrastrip variability, the OneDraw strip was divided into 6 regions and a single 3 mm punch was taken from each region. For the Whatman 903 variability, 3 3mm punches were obtained from each blood spot.

RESULTS

Four remote sampling blood collection devices (**Figure 1**) were assessed. All devices required a separate alcohol swab as well as post acquisition dressing. All but the OneDraw required a separate lancet for skin puncture. Gross metrics show that all samplers yield comparable data (**Figure 2**). As such, fit for purpose utility is a reasonable selection factor for these devices. While the Mitra and Captainer must be processed in their entirety, the OneDraw and paper cards can be partially processed. We assessed the inter- and intra-spot variability on paper cards and found the results to be comparable (**Figure 3**). This is expected as the overall spot radiates outward from the point of deposition no more than 3-5 mm. The OneDraw, which collected blood at one end of a 2 cm strip, seemed to have sufficient length to have chromatographically induced differences in analytes; additionally, there seems to be a thickness change that occurs over the strip such that punches near the terminal end contain more blood than those at the inlet end (**Figures 4, 5, 6**). So, while punches are acceptable for paper cards, it is recommended that OneDraw strips either be processed in their entirety or bisected along the inlet to terminal axis.

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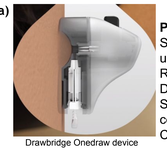
REMOTE SAMPLING TECHNOLOGY COMPARISON

(a)



Drawbridge Onedraw device

Price: $$$ **Comfort**: ❤️❤️❤️ **Volume**: 🪣🪣🪣 **Training**: 🧠

Pros:
Self-contained sampling and storage unit
Reduced discomfort
Defined volume
Storage matrix is Whatman 903 comparable
Controlled Humidity for Drying

Cons:
Single use
Expensive ($10-$20)
Bulky (difficult to pack)
Does not aliquot well (2 strips)
Must dry before processing (4h)

Field Deployment Consideration:
Minimal Training Required
Bulky/difficult to pack
Requires exposure to Arm or Thigh (difficult in BattleDress)
Does not impact hand/finger usage
Durable post-sample packaging

(b)

Whatman 903 ProteinSaver Card

Price: $ **Comfort**: ❤️ **Volume**: 🪣🪣 **Training**: 🧠🧠🧠

Pros:
Gold standard as neonatal blood collection device Well studied paper matrix
Very low cost

Cons:
Difficulty in collecting reproducible blood spots
Separate lancet and storage bags required
Not volumetric
Must be dried before storage

Field Deployment Consideration:
Compact solution, easy to pack
Lancet use requires some training
Hands/Fingers are more accessible in BattleDress, but, may impact hand use.

(c)

Neoteryx Mitra Collection Kit

Price: $$ **Comfort**: ❤️ **Volume**: 🪣 **Training**: 🧠🧠

Pros:
Volumetric with various tip volume options
Easy to collect samples reproducibly

Cons:
Less studied sorbent matrix
Unknown long-term analyte stability

Field Deployment Consideration:
Lancet use requires some training
Hands/Fingers are more accessible in BattleDress, but, may impact hand use.
Sampler and carrier are not packable directly

(d)

Captainer qDBS card

Price: $$ **Comfort**: ❤️ **Volume**: 🪣 **Training**: 🧠🧠🧠

Pros:
Based on Ahlstrom grade 222 paper
Success/Failure visually obvious.
Volumetric acquisition (+/- 0.5 uL)

Cons:
Introduces contaminant signals for the dye and the PVA
Separate lancet required
Requires excess blood for effective capillary action.
Unknown long-term analyte stability

Field Deployment Consideration:
Lancet use requires some training
Hands/Fingers are more accessible in BattleDress, but, may impact hand use.
Easily Packable

Figure 1: Overview of selected remote sampling technology/devices. Panels (a) thru (d) represent four different collection devices for skin puncture blood draws. The OneDraw(a) incorporates a lancet in the collection device while the Papercards(b), Mitra(c) and Capitainer(d) require a separate lancet. The OneDraw requires the collected sample to be moved to a separate container post acquisition while the paper card, Mitra and Capitainer do not. All devices require a separate alcohol swab prior to collection as well as post acquisition care (e.g. band-aids).

GLOBAL UNTARGETED METABOLOMICS DIFFERENCE BETWEEN DEVICES

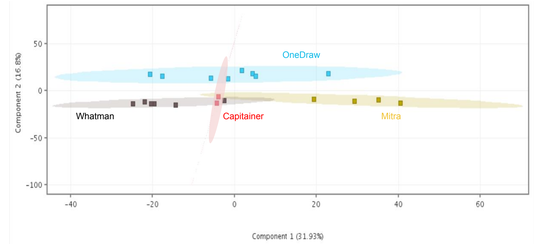
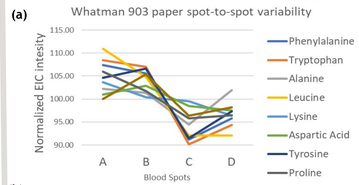
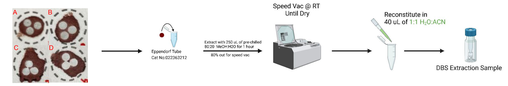


Figure 2: Global difference in blood collection devices via untargeted metabolomics. Whatman 903 paper, Capitainer card, OneDraw, and Mitra devices were used to collect blood from the same volunteer within 30 min. Untargeted feature extraction and normalization was performed and the modality groups are labeled in this PCA plot. Noted is the minimal separation and group overlap.

WHATMAN 903 PAPER INTRA-SPOT VARIABILITY

(a)





(b)

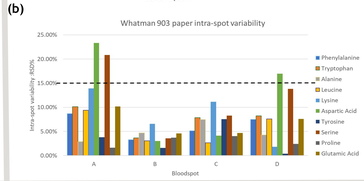
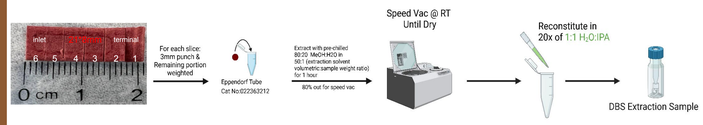


Figure 3: Amino acid retention variability on Whatman 903 paper. 4 blood spots collected from the same volunteer at the same time on a Whatman 903 card. Each whole spot had three 3mm diameter hole punches taken from within the blood spot area. Spot-to-spot variability (a) as well as intra-spot spreading behavior (b) for amino acids (color coded) are shown. Metabolomics measurements were taken on each punch and a targeted analysis was performed to evaluate the level of amino acids within each. In (a), normalization is performed for each species to the average peak area across the different spots. In (b), the black dashline highlights the clinical acceptable variability of 15%.

ONEDRAW STRIP HETEROGENEITY EVALUATION



Onedraw strip ‘punching after slicing’ strategy. Two onedraw devices were sampled on the same volunteer at the same time, one per arm. For sample handling, the Onedraw strip (21*8mm) is sliced into 6 slices and then a 3 mm punch was acquired from each slice to ensure no overlap in punch positions along the strip long edge. The 3 mm sub-punches and slice remaining portions were weighed individually for both extraction solvent volume determination and data normalization purposes. Pre-chilled 80:20 MeOH:H2O was added at a volume 50x of the sample weight value to take extraction solvent & paper matrix partitioning effect into account. After one hour, 80% of the extracted sample was transferred to new eppendorf tube for drying under vacuum centrifugation. For reconstitution, 1:1 H2O:IPA was selected for its optimal recovery performance across the metabolome polarity space. The dried sample was reconstituted back into 20x of the estimated blood volume based on a strip segment “volume” of 12.5 uL – 3 mm punches getting 63 uL and the remainder of the strip getting 187 uL.

Saturated blood Strip Weight Normalization Strategy

Leucine retention profile restoration after slice remaining weight normalization

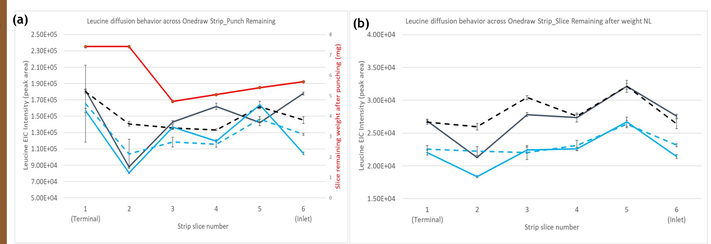


Figure 5: Normalization using strip sample weight (slice punch remaining) restores the retention behavior observed using 3mm sub-punches. The slice remaining portion were weighed before extraction. Assuming sampled blood fully wets the strip, the leucine EIC profile before (a) and after (b) weight normalization.

Amino Acid Retention Behavior Across Onedraw Strip

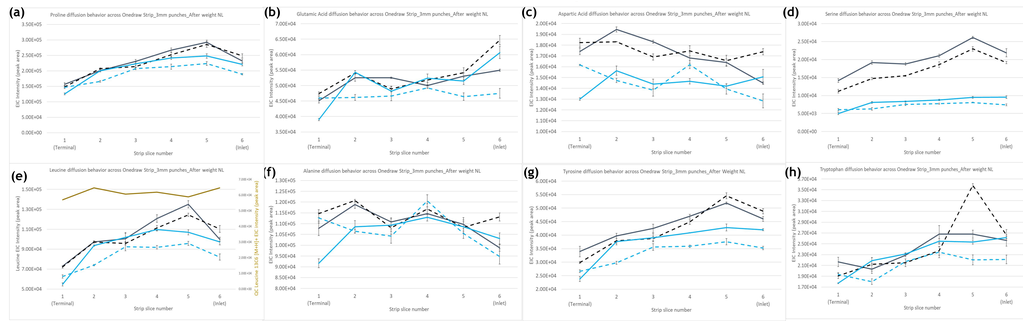


Figure 4: Amino acids retention behavior across the Onedraw strips. Amino acids (AAs) of different properties were included. Charged and polar AAs: Proline (a), Glutamic Acid (b), Aspartic Acid (c), Serine (d); Non-polar AAs: Leucine (e), Alanine (f), Tyrosine (g), Tryptophan (h). Two devices are color coded (#1/#2) and the upper/lower strips are solid/dash line coded. The EIC peak area is normalized to the 3mm punch weight to take matrix heterogeneity into account. L_Leucine d7 $[M+H]^+$ peak areas from QC sample runs are plotted on top of the leucine data (e) to show no drift in instrument performance over the sample table acquisition.

Phospholipid retention profile after 3 mm punch weight normalization

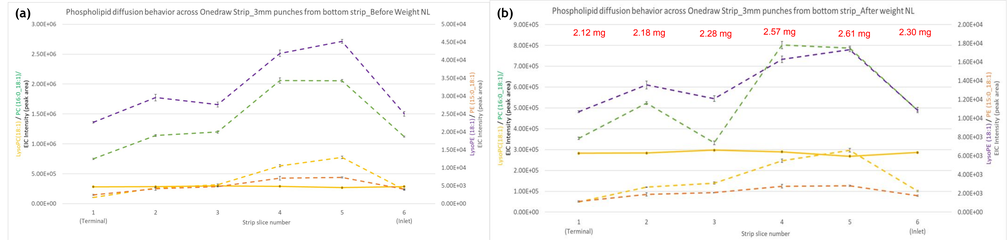


Figure 6: Phospholipid retention behavior across the Onedraw strip. The EIC peak area of LysoPC (18:1), PC (16:0_18:1), LysoPE (18:1), and PE (15:0_18:1) across the 6 slice positions from a bottom strip sample are plotted (dashed lines) together with the LysoPC(18:1)d7 EIC peak areas (solid line) from QC injections planned in between sample runs. (a) is pre-weight normalization retention profile, LysoPC(18:1)d7 peak areas from QC sample runs are plotted together to demonstrate no drift in instrument performance. (b) is post normalization retention profile using 3mm punch weights which are marked on top of each slice position.