•Dried blood Spot ● LC-MS ● Untargeted metabolomics

Evaluation of Metabolomics of Dried Blood from Various Remote Sampling Technologies: Parameters of Acquisition, Extraction and Analysis.

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Background

Capillary blood acquisition allows for blood acquisition methods that can be self-administered. Combined with drying (such as on paper), to remove the need for a "cold chain", this represents the most convenient strategy towards "remote sampling" and biobanking of biological samples. Commercial devices feature different matrix materials and sampling technologies, often targeting different sampling locations (e.g. finger) and sample volumes for different application scenarios.

Here, the homogeneity of the dried sample obtained along with the capability to recover sample reproducibly and efficiently from the matrix are critical to deliver multiplexed and quantitative analytical measurements downstream. In this project, the practicality of sub-aliquoting (enabling banking and multiple analyses), sample extraction, sample homogeneity and sample handling are assessed to evaluate their suitability as biobanking/screening devices and identify real-world challenges and opportunities.

Highlights

- Multiple DBS acquisition/storage technologies are shown to be comparable with regards to observed features – there are some noted differences.
- OneDraw and Mitra acquisition can be performed with minimal training, however, skin puncture acquisition to paper cards requires greater training and practice to perform.
- Sample sub-aliquoting feasibility is well studied for the selected devices. Post acquisition fractionation is optimal from paper cards and is limited on OneDraw devices and non-existent on Mitra VAMS devices.

Evaluation Strategy

A DBS QC panel was used that included Cambridge Isotope Laboratories (CIL) Metabolomics QC1 & QC2 kit, Avanti Splash mix kit, and individual metabolites to cover the m/z and polarity range of the metabolome.

A HILIC method (Figure 1(a)) and RPLC method (Figure 1(b) & 1(c)) were then optimized for the DBS QC panel and the possible adduct ion formation for each DBS QC panel species was performed on an Agilent 1290 InfinityUPLC system hyphenated with TIMSTOF Pro2 mass spectrometer equipped with VIP-HESI source.

Sample blood volume estimation is carried out to address the sub-aliquot heterogeneity using either the relative weight or the relative area with the device claimed sampling volume as reference: 75-80 µL per whatman spot, 20 µL per Mitra Tip, and 75 µL per Onedraw Strips. All sample weights were measured on scale prior extraction, with Onedraw strip grid relative area ratios also captured post-extraction.

For pre-analytics workflow, the sample weight and estimated blood volume are used to determine extraction solvent volume and reconstitution solvent volume to ensure constant dilution factor across samples.

Results

Remote sampling technologies are emerging as efficacious and robust methods to enable blood sample acquisition without clinical intervention and often self-acquired. Within this realm, our experiences are that paper card acquisition requires significant training and practice to perform effectively. In contrast, the Mitra VAMS devices require some training and the OneDraw require only minor training (Figure 7).

The strongest advantage we found for the paper cards is that they allow multiple sampling from one card with minimal differences. We conclude that OneDraw strips can be bisected allowing for 4 subfractions to be produced per draw – however, we have not yet evaluated the draw to draw variability. In contrast, Mitra VAMS devices are neigh impossible to fractionate – that said, it is easy to acquire 4 samples from a fingerstick. Our concern here is that the VAMS polymer has not been evaluated for longer term storage.

We do note that most features are consistent across point of acquisition and sampling technology used. That said, we do note a couple "exertion" features that do seem to differentiate based on location of acquisition. In particular lactate and hypoxanthine seem to be elevated in shoulder proximal draw as compared to fingerstick draw.

We conclude that it is reasonable to allow price point and parameters of acquisition to dictate the methodology chosen. For in lab sampling, Mitra VAMS has high utility, for maximal flexibility and price point, paper cards have high utility. For least amount of training/handling and storage concerns, OneDraw has a strong advantage in the methodologies studied here.

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Disclosure:
The authors have no conflicts of interest to declare.

Remote Sampling Technology Comparison & Evaluation Strategy

(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(less than $1 per card)

Cons:
Difficulty in collecting reproducible blood spots
Separate lancet/storage bags required
Not volumetric
Training is required

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**: 🧠🧠

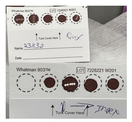
Pros:
Volumetric with various tip volume options
Easy to collect samples reproducibly

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

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

Sample Relative weight/area ratio measured for blood volume Estimation, Extraction Volume Normalization and Data Normalization



1.6, 3, and 6mm sub-punches

20 µL Tips

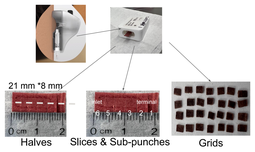
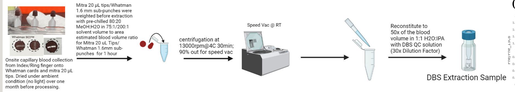


Figure 2: Normalization Strategy for the samples acquired from the selected remote sampling devices. Sample blood volume estimation is carried out using either the relative weight or the relative area with the device claimed sampling volume as reference: 75-80 µL per whatman spot, 20 µL per Mitra Tip, and 75 µL per Onedraw Strips. All sample weights were measured on scale prior extraction, with Onedraw strip grid relative area ratios also captured post-extraction. For extraction workflow, the extraction solvent volume was determined for each sample using a high and fixed extraction solvent volume to sample weight ratio. Then the same percentage of extract supernatant were transferred for speed vacuum drying. The estimated blood volume was later used for calculating reconstitution solvent volume to ensure constant dilution factor across samples.

Sub-Aliquoting Feasibility

Sampling Device Matrix (Paper vs Polymer) Effect Observed for Phospholipids with 80:20 MeOH:H2O as the Extraction Solvent



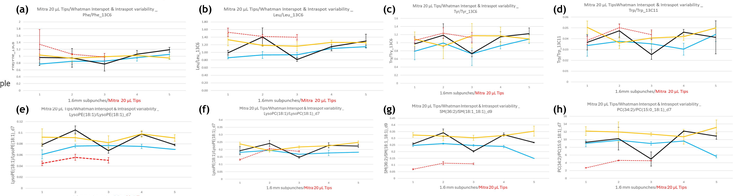
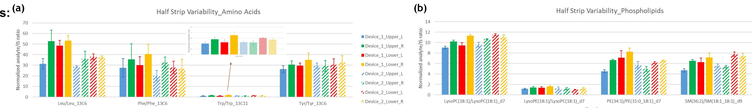


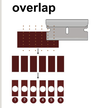
Figure 3: Tip-to-tip variability among Mitra tips and inter/intra spot variability across Whatman Spots for Amino Acids and Phospholipids. The metabolite/Internal Standard ratios measured from three 20 µL Mitra Tips(plotted in red) and across five 1.6mm sub-punches from one index finger spot(plotted in blue) and two index finger spots (plotted in black and yellow) are plotted for the following compounds: (a) Phenylalanine (b) Leucine (c) Tyrosine (d) Tryptophan (e) LysoPE(18:1) (f) LysoPC(18:1) (g) SM(36:2) (h) PC(34:2).

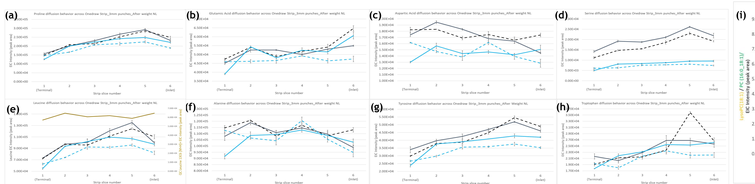
Onedraw sub-aliquoting feasibility: Chromatography Effect Across strip observed for Amino Acids and Phospholipids

Halving strips: are they equivalent?



Punching after slicing to ensure no longitudinal overlap





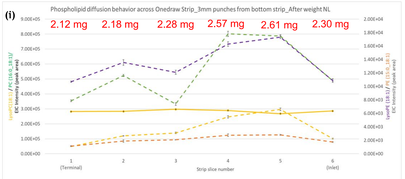
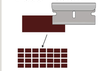


Figure 4: Amino acids retention behavior across the Onedraw strips. Two Onedraw devices were sampled on the same individual at the same time and processed together later after completely dried. Amino acids (AAs) of different properties were included Two devices(1/2, 4 strips) are pattern coded and the Upper_Left, Upeer_Right, Lower_left, and Lower_Right halves are color coded.

Figure 5: Amino acids and Phospholipids retention behavior across the Onedraw strips. 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). The two devices are color coded (#1/#2) and the upper/lower strips are solid/dash line coded. The 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. For phospholipids in (i), 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. LysoPC(18:1)d7 peak areas from QC sample runs are plotted together to demonstrate no drift in instrument performance. All EIC peak areas are normalized to the 3mm punch weight marked on top of each slice position in (i).

Gridding strips for compound over strip distribution visualization



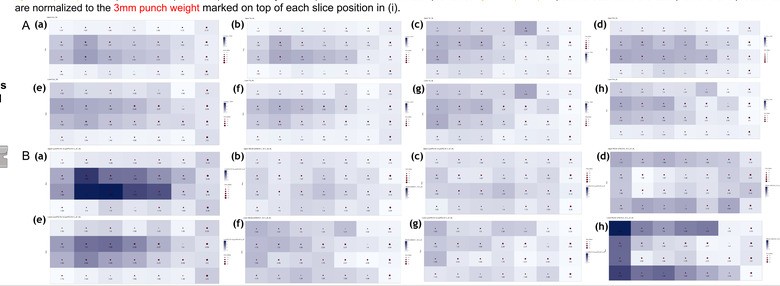


Figure 6: Heatmap view of Amino Acids and Phospholipid retention profile over the OneDraw strips. Panel **A** includes the following amino acids from left to right: Leu, Trp, Tyr, Phe, and panel **B** include the following phospholipids: LysoPC(18:1), SM(36:2), LysoPE(18:1), and PE(34:1). Within each panel (a) - (d) are for the upper strip and (e) - (h) are for the lower strip. 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. For amino acids, the longitudinal chromatography effect are consistent with the punching after slicing experiment; more insights on radial distribution that the center abundance is higher than the edge is obtained. For Lipids, differential chromatography effect were observed for different lipid classes.

Capillary Blood Acquisition Challenges

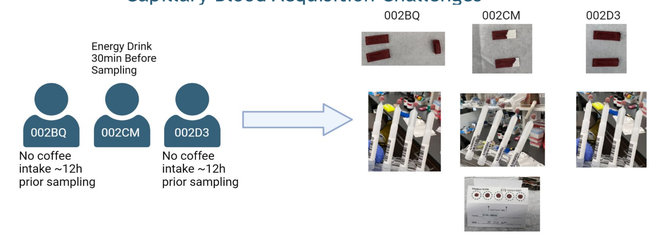


Figure 7: Onsite recruitment of participants for capillary blood sampling using the Onedraw device, 20µL Mitra tips, and Whatman Paper cards. Three volunteers were enrolled: 002BQ, 002CM, and 002D3. 002CM drank one 16 oz can of sugar free Monster and one 16 oz can of sugar free Rip-It energy drink before sampling. The other two had not drink coffee or tea ~12h prior. OneDraw devices were applied first onto participant arms, finger capillary blood were sampled at the same onto Mitra tips and Whatman paper cards. All samples were self acquired. **Neither 2BQ and 2D3 were able to reliably acquire blood spots to paper cards**. The blood volume estimation for the undersampled OneDraw Strip for 002CM was performed using the filled/total relative area ratio calculated with ImageJ. For extraction workflow, the extraction solvent volume was determined for each sample using 75:1 extraction solvent volume to sample blood volume ratio. Then 80% of the extract supernatant were transferred for speed vacuum drying. A 20x dilution factor were applied for calculating reconstitution solvent volume to ensure constant dilution factor across samples.

Known Exposure Sampling Comparison: Arm/Finger

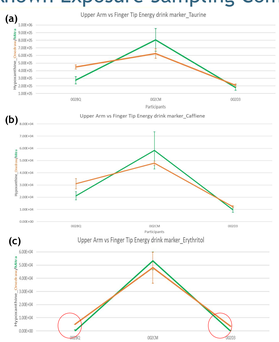


Figure 8: Energy drink marker measurement with Mitra and Onedraw. Consistent EIC profiles for three energy drink Ingredients (a) Taurine, (b) Caffeine, (c) Erythritol (low calories sweetener) across participants are observed using both the Mitra and Onedraw, with 002CM having the highest abundance overall. Interestingly, Erythritol were measured within the onedraw samples but not Mitra for participants 002BQ and 002D3 (circled).

Differential Markers: Site of Acquisition

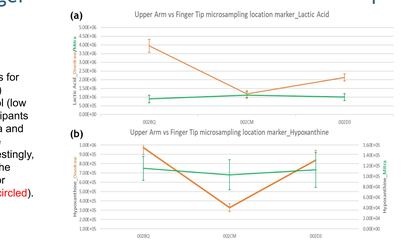


Figure 9: Sampling Location May Affect Some Markers of Interest. While we have seen a strong concordance of signals from individuals independent of location of sampling, we observed that for n=3, markers associated with exercise, such as lactic acid **(a)** and hypoxanthine **(b)** were consistently found to be elevated in the samples taken from the OneDraw (shoulder) vs the Mitra (fingertip).

Paper vs Polymer

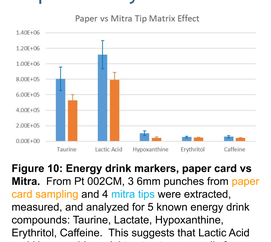


Figure 10: Energy drink markers, paper card vs Mitra. From Pt 002CM, 3 6mm punches from paper card sampling and 4 mitra tips were extracted, measured, and analyzed for 5 known energy drink compounds: Taurine, Lactate, Hypoxanthine, Erythritol, Caffeine. This suggests that Lactic Acid and Hypoxanthine might extract more easily from Mitra tips. When compared to **Figure 9**, this affirms the difference induced from point of sample acquisition.

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