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Incorporating novel synthetic DBS substrates into a blood microsampling device for multi-omics analyses.

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Overview

We present four studies associated with blood microsampling using a Dried Blood Spot (DBS) workflow on paper and polymer substrates:

1. Targeted metabolomics analysis of 2.74 µL blood biological and technical replicates using GC-MS/MS.
2. Comparison of sub-punch and accurate volume DBS for amino acids and acyl-carnitines across of range of hematocrits using FIA-MS/MS.
3. Development of a synthetic polymer DBS substrate for blood derived FAME analysis by GC-MS.
4. Extraction efficiency of DNA from different DBS substrates – a comparison of paper and synthetic polymer.

Figure 1. The hemaPEN® has been designed to collect replicate, volumetrically accurate (2.74 µL) and precise volumes of blood. Shown here collecting a capillary blood sample, it could be used with any blood source. An embedded desiccant is designed to dry the collected sample inside the contained device. The device does not contain a lancet.

Introduction

- Blood microsampling, such as dry blood spotting (DBS) is safe, convenient, "patient centric" and ideal for expansive population health initiatives as well as research-focused "omics" studies.
- DBS adoption has been limited by the perceived lack of volumetric accuracy and the analytical bias associated with variable blood hematocrit (HCT) and analyte extraction from the paper substrate.
- This work introduces a novel device for collecting very small blood volumes (<3 µL) and their subsequent metabolite and DNA analysis of the dried blood extracts from paper substrates as well as a panel of novel porous polymer substrates. The substrates are encapsulated in a blood collection device designed to collect an accurate volume and stored in a secure desiccated environment.

Methods

Study 1

Three biological replicates were collected within the same time point (within 5 min) from a healthy donor by fingerstick capillary blood. Each sample collects 4 x 2.74 µL technical replicates of whole blood onto pre-punched grade PerkinElmer 226™ filter paper. Samples were extracted and analyzed as TMS derivatives by GC-MS/MS. Sample preparation - each technical replicate (12 samples in total) was extracted into 100 µL of chilled methanol (IS at 5 µM); dried and converted to TMS-derivatives. A 1 µL sample was injected into a Shimadzu GCMS-TQ8040 (utilizing Shimadzu Smart Metabolites Database). Data was processed using Shimadzu's Lab Solutions software, and the matrix output was then imported into Metabo Analyst for statistical analysis. Log transformation and median normalization (with no scaling) was applied as the data pre-treatment step in order to reduce the differences between possible metabolite concentrations and influence of other factors such as measurement noise.

Study 2

Preparation of blood - five different HCT levels were prepared from a single donor. Eight hemaPEN were used to collect and dry samples at each HCT level using the accurate volume workflow (n = 32 pre-punch Whatman™ 903 specimen filter paper). DBS cards (Whatman™ 903 specimen filter paper – 4 circles each containing 75 µL of the prepared blood) were used for the sub-punch workflow where 4 × 3.2 mm sub-punches were taken from each of the blood spots (n = 32 sub-punch DBS). All DBS were extracted with 150 µL of 100% MeOH containing the appropriate deuterated standard; shaken for 60 min and dried under an air stream at 65°C then reconstituted in 200 µL of 70% MeCN. 10 µL samples were flow injected using the Acquity UPLC (SM-FTN) into a Waters Acquity TQ MS detector. Twenty of the extracted metabolites (amino acids and acyl-carnitines) were analysed and reported in µmol/L based on the peak ratio of the extracted analyte and corresponding deuterated IS. Data analytics on the µmol/L peak ratios was performed using SIMCA-14 (Sartorius Stedim Data Analytics).

Study 3

10 µL and 3 µL blood samples were applied to both 226 paper substrate (6 mm diameter) as well as the synthetic polymer mPPM inserts. Samples were dried and added to 2 mL of 1% (v/v) H_2SO_4 in anhydrous methanol. Transmethylation reaction was performed at 70°C for 3 h. FAMEs were extracted into 600 µL of n-heptane and analysed by GC-MS (Thermo Scientific FOCUS GC with DSQ II MS). Whole blood was also transmethylated as described above and used as the reference for all DBS workflows.

Study 4

Five different paper substrates and the mPPM insert were used to explore the extraction performance using two different commercial kits Qiagen QIAamp DNA and Zymo Quick DNA mini. Four x 3 mm sub-punch samples were collected from each card and a 12 µL blood sample was collected onto the mPPM insert. All samples were incubated overnight in PBS and Proteinase K solution prior to extraction. DNA concentration was measured by Qubit Fluorometric Quantitation and high molecular weight quality determined by agarose gel electrophoresis.

Study 1

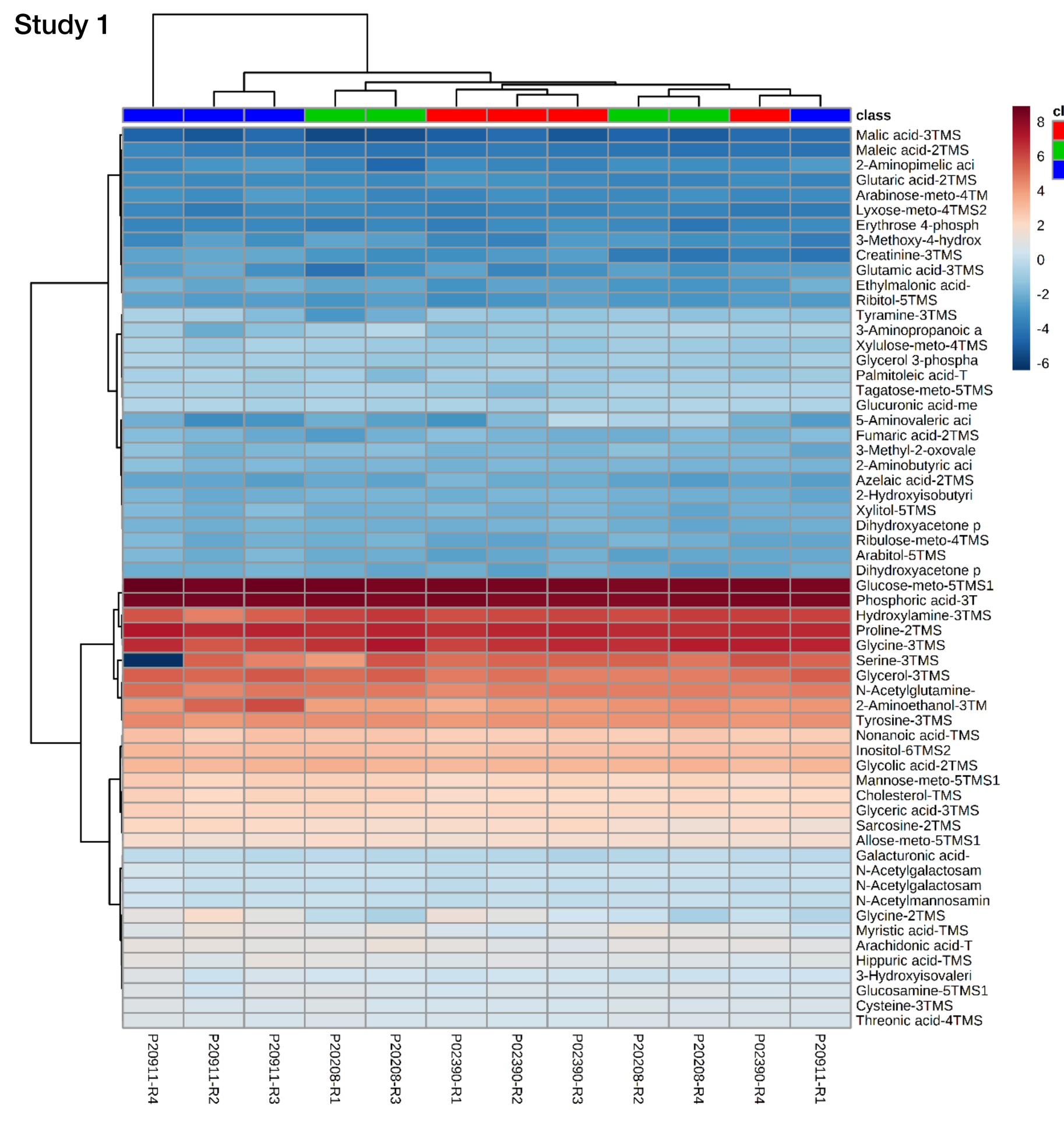

Figure 2. Two-way heat map displaying the normalized peak intensities for the 50 most intense TMS derivatized metabolites from 3 biological replicates (3 hemaPEN labelled Red (P02390), Green (P20208) and Blue (P20911)) and 4 technical replicates (each cell).

Conclusion:

- hemaPEN generates true technical replicates from the same blood sample (4 x 2.74 µL).
- Ideal sampling strategy for metabolomics studies - no significant fold (2-fold)/p-value (0.05) differences for any of the biological or technical replicates.

Study 2

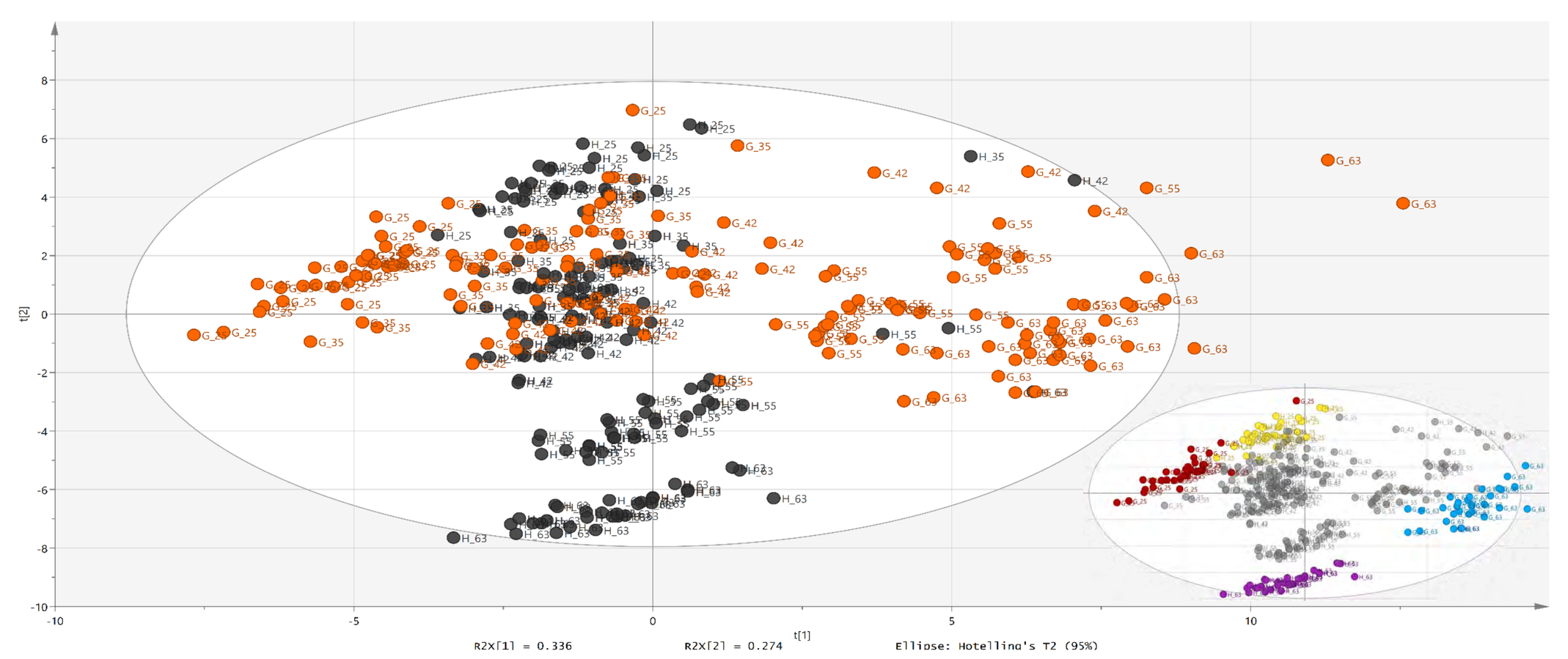

Figure 3. PCA Score Plot of all 20 amino acids & acyl-carnitines peak ratios for accurate volume pre-punched DBS (Grey) and sub-punch DBS (Orange) (n=32 for each of the 5 HCT and each workflow).
PC1 - Observed variation attributed to analytes influenced by the sub-punch workflow.
PC2 - Observed variation attributed to analytes influenced by the HCT of the accurate volume samples. Inset shows the same PCA score plot highlighting the volumetric bias at low and high HCTs for sub-punch workflows compared to the accurate volume hemaPEN® sampling.

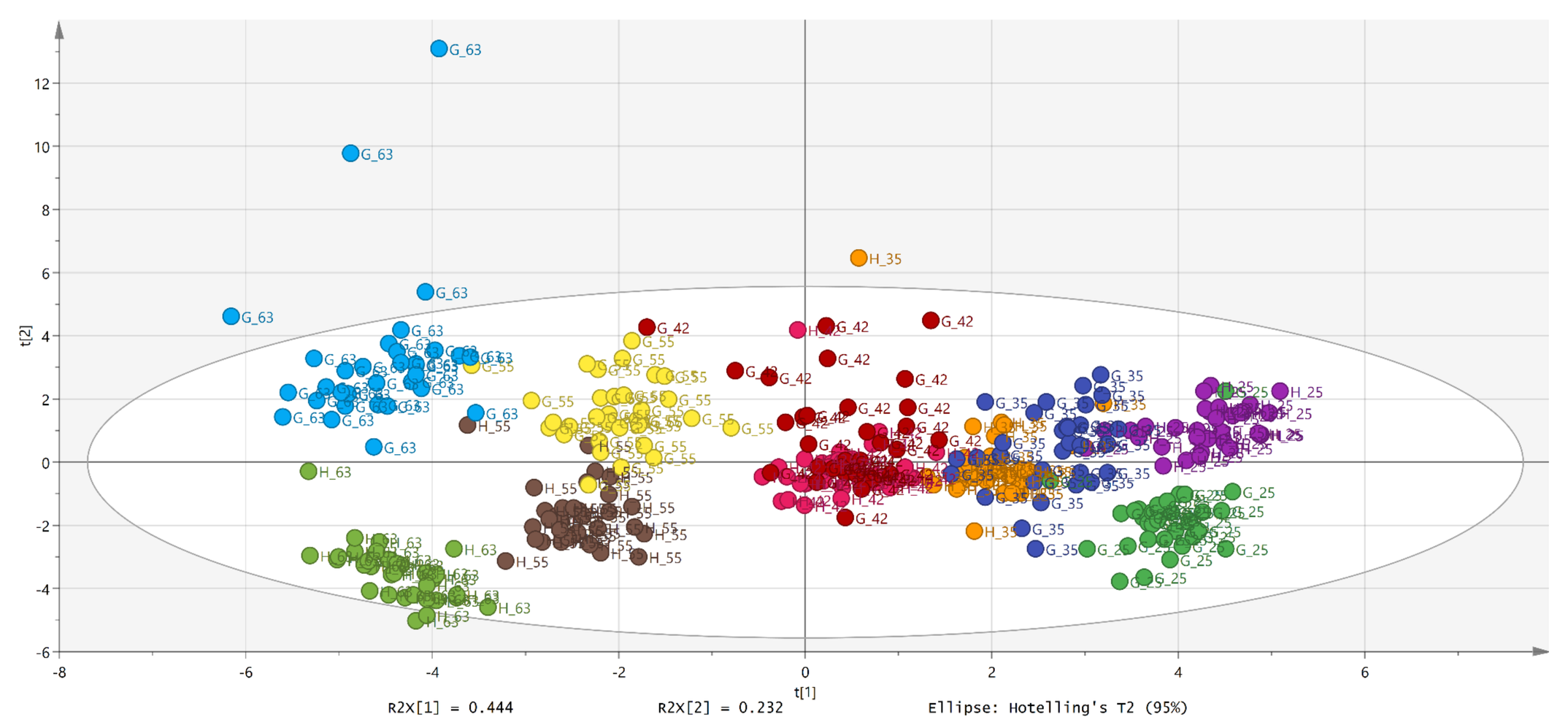

Figure 4. PCA Score Plot of all acyl-carnitine peak ratios for accurate volume pre-punched DBS (H-series) and sub-punch DBS (G-series) workflows for five different HCTs (n=32 for each HCT and each workflow). PC1 accounts for 44.2% of the observed variation which can be attributed to the HCT regardless of the DBS workflow. PC2 accounts for 23.2% of the observed variation which can be attributed to the different DBS workflows – both the sub-punch and accurate volume are well separated at 63%, 55% and 25% HCTs.

Study 3

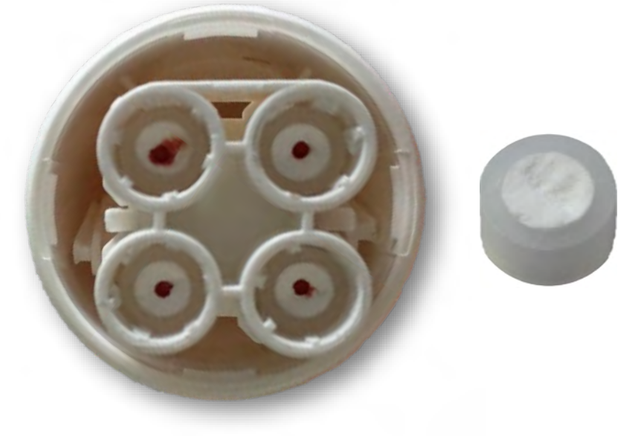

Figure 5. hemaPEN cartridge with mPPM inserts (3.6 mm ID x 3.5 mm depth) and 2.74 µL capillary blood collection.

Substrate contamination of SFA

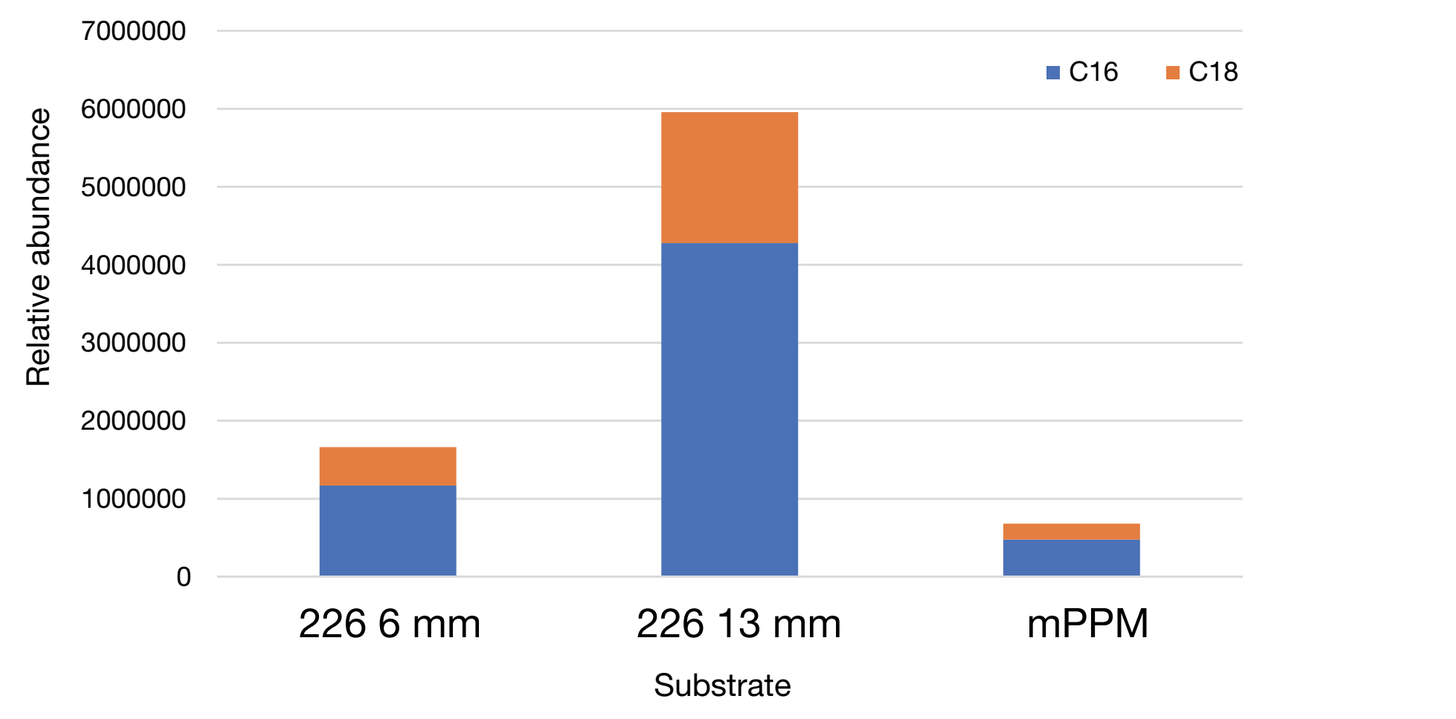

Figure 6. Relative abundance of the saturated fatty acid contamination to different DBS substrates.

Conclusion:
Here we have characterized the contribution different substrates have on the DBS workflow for the measurement of FAME and the significant contribution of saturated fatty acids in the paper substrate. The synthetic mPPM substrate has a negligible SFA contribution and the mPPM insert for the hemaPEN is ideal for collecting blood volumes from 2.74 – 25 µL.

Table I. Each class of FAME (n=3) reported as % contribution to the total summed peak area for all FAME (taking into account the relative response of each FAME to the 37 FAME standard). Colored cells represent a difference of >10% from the blood reference.

3 µL	Blood	226 - 6 mm	mPPM
SFA	29.4±0.4%	34.0±0.9%	29.6±0.7%
MUFA	18.3±0.5	18.0±0.4%	19.1±0.2%
n-6 PUFA	42.5±0.3%	39.0±0.6%	41.7±0.3%
n-3 PUFA	8.3±0.2%	7.6±0.4%	8.3±0.3%

10 µL	Blood	226 - 6 mm	mPPM
SFA	27.7±0.3%	31.9±0.6%	28.0±0.4%
MUFA	18.2±0.2%	18.4±0.5%	18.2±0.1%
n-6 PUFA	44.7±0.6%	41.2±0.2%	43.9±0.5%
n-3 PUFA	8.0±0.1%	6.9±0.1%	8.1±0.1%

Contribution of substrate contamination to % FA measurement

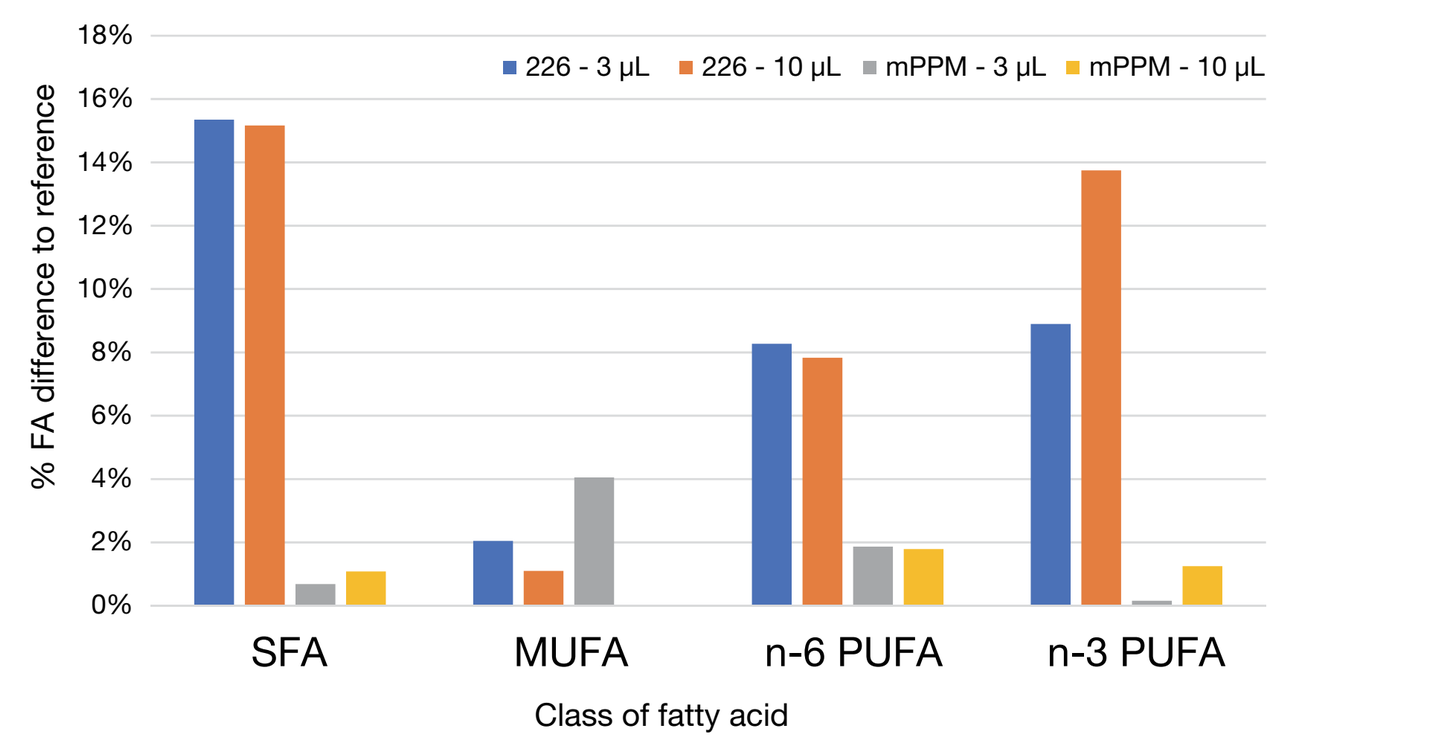

Figure 7. Impact of substrate contamination on the relative abundance of blood sourced fatty acids.

Study 4

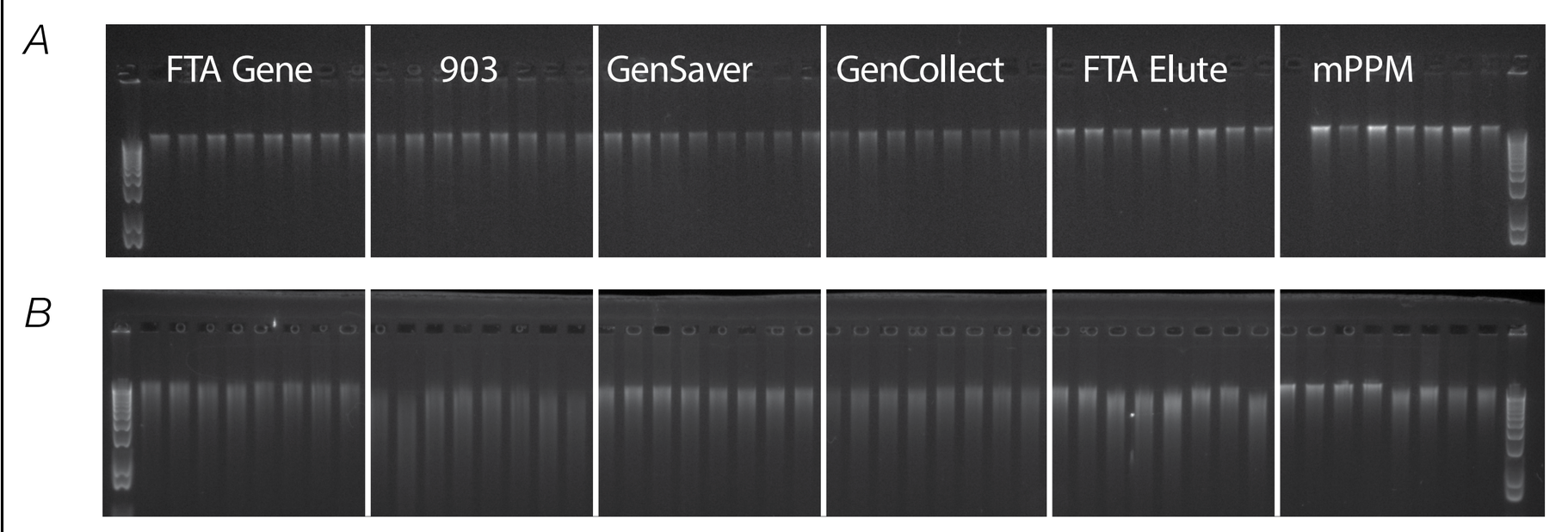

Figure 8. 1% Agarose gel electrophoresis of DNA extracts from different DBS paper substrates and mPPM insert. DNA extracts were prepared from A – Qiagen QIAamp DNA and B - Zymo Quick DNA mini.

Total yield extracted for each kit and substrate

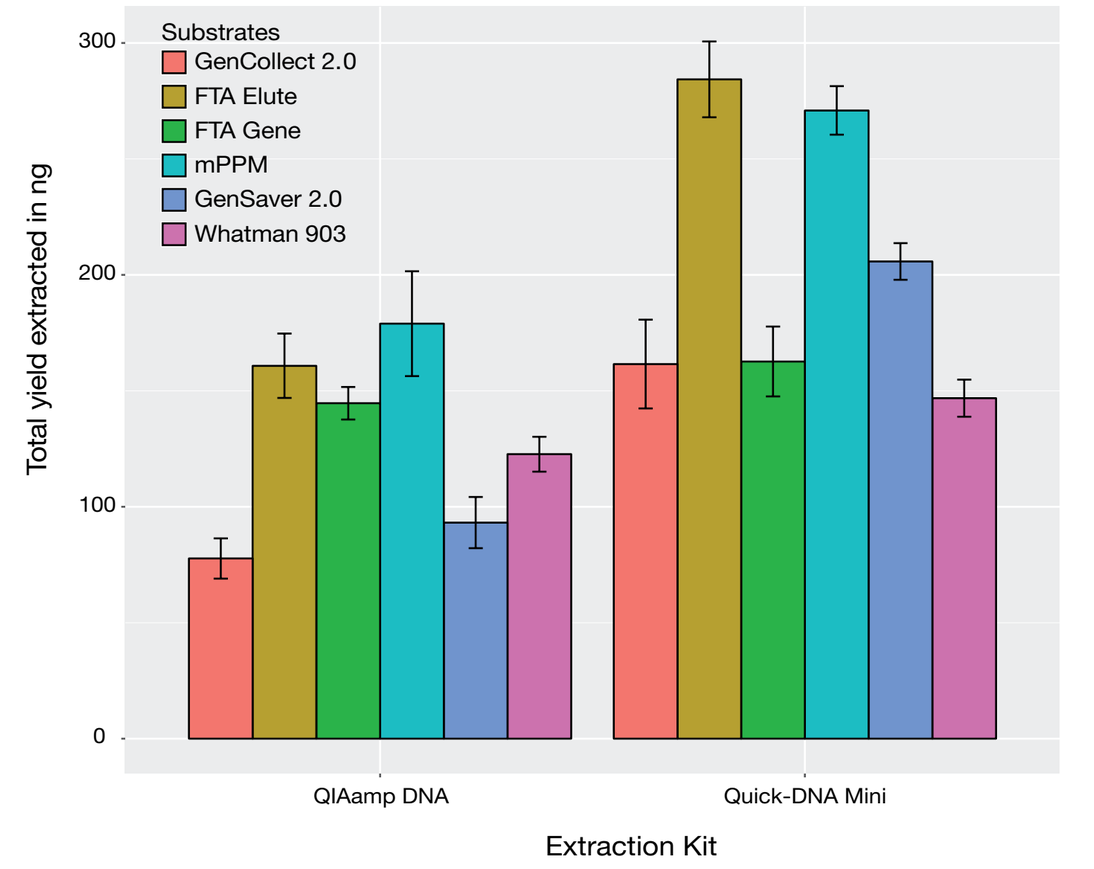

Figure 9. DNA recovery from six different substrates and two different DNA extraction kits.

Conclusion:
All of the DBS substrates resulted in recoveries of DNA although the FTA Elute and mPPM consistently outperformed the other substrates both for yield and high molecular weight quality.

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