Can volumetric absorptive micro sampling in the presence of a stable-isotope labeled protein standard control pre-analytical variability in proteomics?

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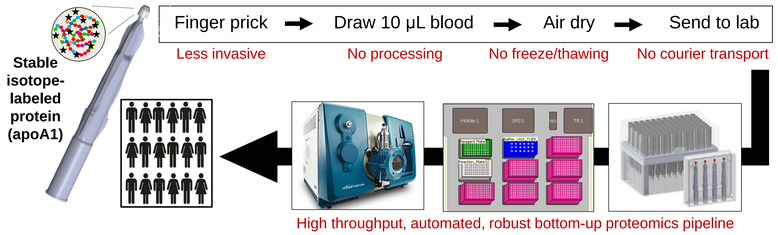
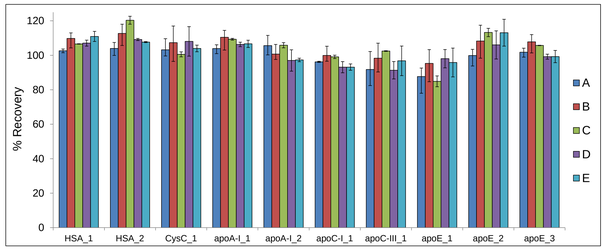
Overview



The easy collection, storage, and mail shipment of (at-home collected) blood makes volumetric absorptive microsampling (**VAMS**) attractive for **population-based biomarker screenings**. The **(pre-)analytical variation** is minimized by inclusion of a stable-isotope labeled protein standard at the time of sample collection and by full-automation of the bottom-up proteomics workflow.

Introduction

VAMS accurately collects a fixed small volume (10 µL) of blood, and overcomes hematocrit bias and sample heterogeneity issues associated with dried blood spots [Denniff and Spooner, 2014]. We have optimized the extraction of proteins with focus on compatibility with automation and upstream processes, *i.e.*, denaturation, reduction (**Fig. 1**), and trypsin digestion (**Fig. 2**).



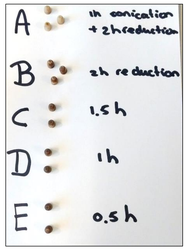


Figure 1: The recommended sonication (**A**) limits automation, whereas rigorous shaking (1200 RPM) at 60ºC during denaturation (2% OGS) and reduction (TCEP) provides extraction recoveries of 94-120% (**B-E**) and allows integration into a standardized proteomics workflow.

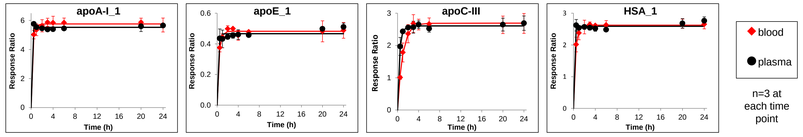
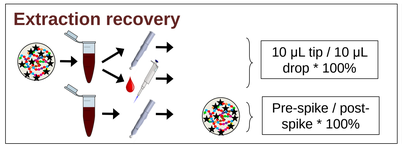
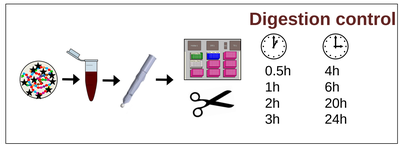
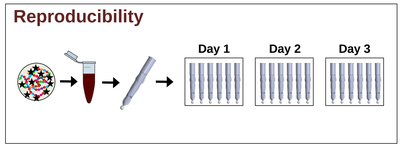


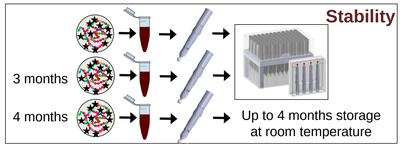
Figure 2: Trypsin digestion efficiency of proteins in blood was identical to plasma.

Methods









Results

Extraction recovery

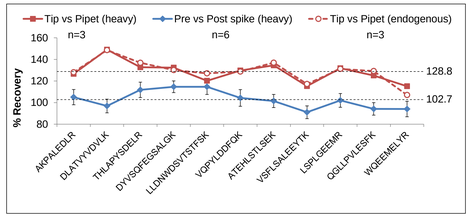


Figure 3: The average extraction recovery of heavy apoA1was 102.7% when compared to post-spike response. Despite the over-recovery of heavy and endogenous apoA1 (average 128.8%) when compared to 10 µL blood, recoveries of heavy and endogenous peptides vary concordantly, indicating normalization of heavy apoA1 for variations in extraction efficiency.

Reproducibility

Table 1: Within-run and between-day reproducibility of the automated workflow for extraction, digestion and LC-MRM analysis of apoA1 with a stable-isotope labeled protein (spiked into blood) or peptide (spiked before digestion) standard.

Peptide	IS	Ratio Day1 (n=6)	Ratio Day2 (n=6)	Ratio Day3 (n=6)	Average	Daily means CV (%)	Average within-run CV (%)	Between-Day CV (%)	Total CV (%)
AKPALEDLR	heavy apoA1	8.2	8.3	8.4	8.3	1.2	2.2	0	2.2
ATEHLSTLSEK	heavy apoA1	8.5	8.4	8.5	8.5	0.5	4.2	0	4.2
DLATVYVDVLK	heavy apoA1	8.2	8.3	8.2	8.2	0.6	1.6	0	1.6
DYVSQFEGSALGK	heavy apoA1	7.7	7.9	7.8	7.8	0.9	1.3	0	1.3
LLDNWDSVTSTFSK	heavy apoA1	8.4	8.1	8.0	8.2	2.0	1.7	1.9	2.6
QGLLPVLESFK	heavy apoA1	8.7	8.6	8.7	8.7	0.9	1.8	0.6	1.9
VQPYLDDFQK	heavy apoA1	8.0	8.1	8.2	8.1	1.3	3.2	0	3.2
VSFLSALEEYTK	heavy apoA1	8.8	8.7	9.1	8.9	1.9	2.1	0	2.1
THLAPYSDELR	heavy apoA1	8.4	8.5	8.5	8.4	0.5	3.1	0	3.1
THLAPYSDELR	heavy peptide	4.8	3.4	4.5	4.2	17.6	8.8	17.2	19.3

No variability from sampling, preparation of standards Reduced variability from extraction, digestion

Includes variability from sampling, preparation of standards (between-days), extraction, digestion

Digestion control

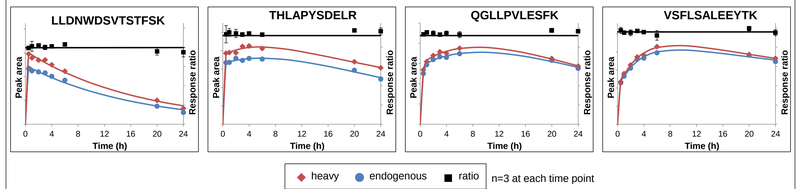


Figure 4: Although different peptides from apoA1 show different trypsin digestion kinetics, the formation and degradation of endogenous and heavy apoA1 occur concordantly, indicating normalization of heavy apoA1 for variations in digestion efficiency.

Stability

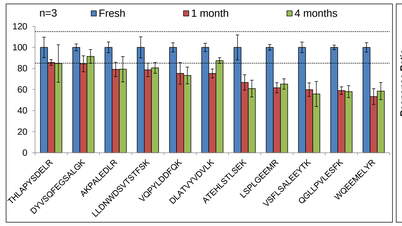


Figure 5: Comparison of heavy apoA1 peptide responses from freshly prepared tips with tips stored for 1 or 4 months.

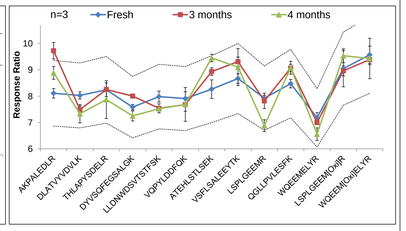
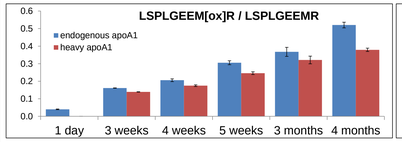


Figure 6: Comparison of apoA1 light-to-heavy peptide ratios from freshly prepared tips with tips stored for 3 or 4 months.



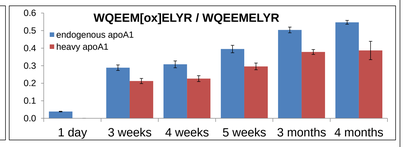


Figure 7: Oxidation ratio of two Methionine-containing peptides from endogenous and heavy apoA1 during storage of VAMS tips.

Conclusions

- **Volumetric absorptive microsampling** is applicable to **proteomics** studies
- **Automation** enables the required **robustness** and **throughput** (within-run %CV <5.0%)
- Addition of a **stable isotope labeled protein** (apoA1) further improves reproducibility (total workflow %CV < 5.0%) and furthermore allows **normalization** and **quality control** for
  - **extraction** recovery
  - **digestion** efficiency
  - sample **storage**
- Future studies are planned to evaluate the effectiveness of heavy apoA1 pre-loaded onto tips