Advancing blood proteome analysis past the plasma age: Mass spectrometry of whole blood collected via volumetric absorptive microsampling devices (VAMS)

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BACKGROUND

Plasma: the current state of affairs

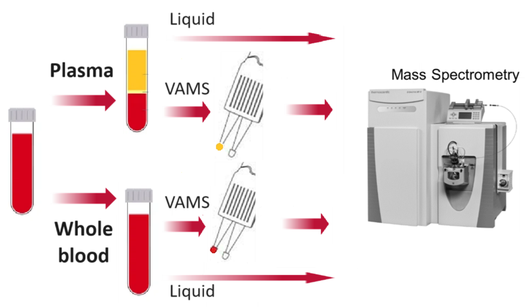
Approximately 40% of clinical tests rely on plasma analysis, however, mass spectrometry-based biomarker discovery using plasma is stymied due to being comprised of 22 highly abundant proteins that limit the detection of low abundance proteins. In addition, the very processing steps required to isolate plasma, such as anticoagulant use and centrifugation, can introduce significant variability into the results and adds additional collection and processing requirements which can be a logistical challenge in larger studies. In addition, obtaining plasma requires in-clinic venous blood collection, which is expensive and logistically difficult and severely limits the number of samples. Longitudinal plasma sampling is even more limited.

Whole blood and VAMS: the potential solution

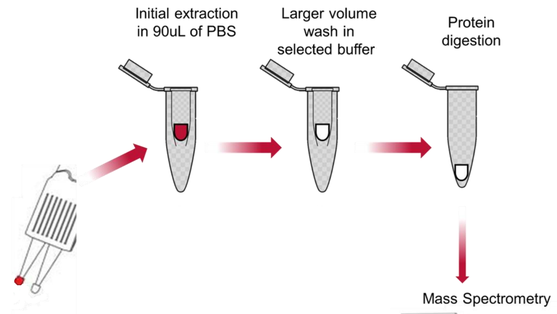
At-home collected fingerprick whole blood, using volumetric absorptive microsampling (VAMS) devices, is an attractive alternative to plasma as it requires no processing and can be collected longitudinally in a patient-centric manner. However, whole blood does have a very wide dynamic range. Increased focus on microsampled blood using VAMS has driven the need to develop sample preparation methods that enable whole blood analysis. Our datasets show that sequential extraction, washing and in-situ digestion in VAMS devices can overcome the dynamic range issues of blood. These methods have minimal processing steps, in 96 well format, and increase the number of detectable proteins with low CVs[1].

METHODS

1. Liquid vs VAMS



2. Optimised VAMS method



Plasma processing methods: Whole blood collected into various vacutainers and plasma/serum produced by centrifugation at 1500g for 10mins. Two different centrifuges tested as well as effect of centrifuging once (single spun) or twice (double spun).

VAMS storage: VAMS samples either processed immediately or stored for 3 months at RT, 4°C, -80°C. Samples were stored either immediately after collection or after washing.

Mass spectrometry: Proteins digested with trypsin (1µg) overnight. Peptides separated using a one-hour gradient and detected using a HF-X Orbitrap mass spectrometer in DIA-mode.

RESULTS

PLASMA

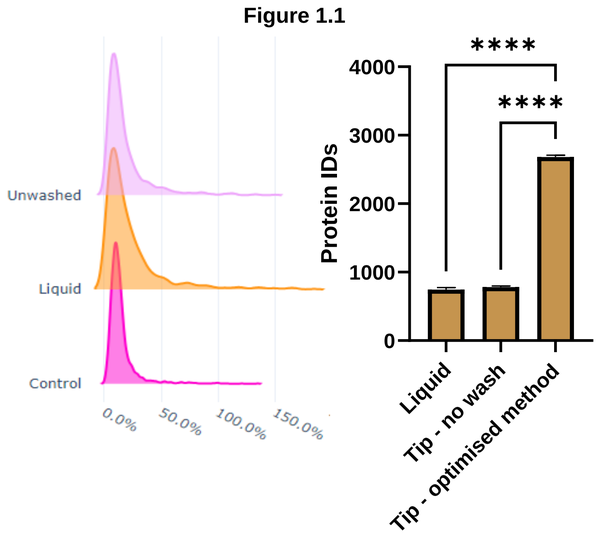
1. Comparison of liquid samples to VAMS samples

Figure 1.1: Number of proteins identified in plasma for liquid samples compared to two VAMS methods and resulting %CV (n=4).

Plasma was produced by centrifuging EDTA anticoagulated whole blood. Liquid plasma samples were prepared by lysing 1µL of sample in 100µL lysis buffer before being digested and analysed. For VAMS samples, 30µL plasma was applied to a Mitra tip and dried. Samples were processed according to the optimal VAMS protocol (control) or the optimal VAMS protocol without the initial 2 washes (unwashed).

There was no significant difference in the number of protein IDs between the liquid samples and the unwashed VAMS. Our optimised VAMS method increased protein identifications by 3.5-fold and %CVs were reduced from 13.8% to 11.5%.

Figure 1.1



WHOLE BLOOD

Figure 1.2

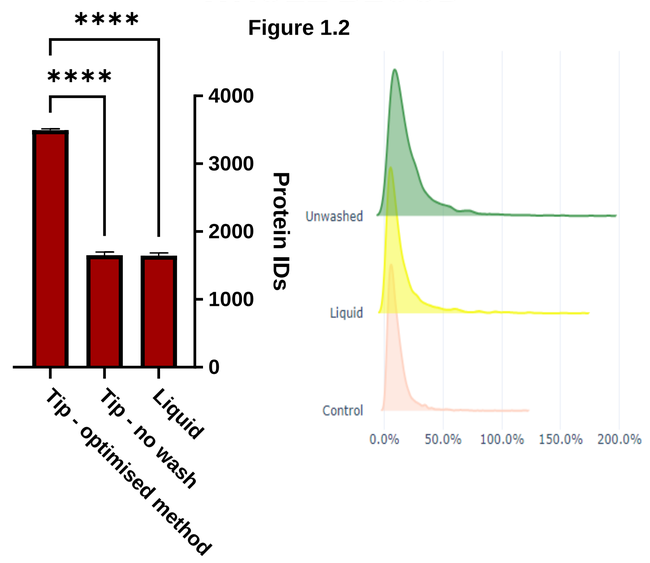


Figure 1.2: Number of proteins identified in whole blood for liquid samples compared to two VAMS methods and resulting %CV (n=4).

Whole blood was collected into an EDTA vacutainer. Liquid whole blood samples were prepared by lysing 1µL of sample in 100µL lysis buffer before being digested and analysed. For VAMS samples, 30µL whole blood was applied to a Mitra tip and dried. VAMS samples were processed as per the plasma VAMS methods.

Similarly to plasma, there was no significant difference in the number of protein IDs between the liquid samples and the unwashed VAMS. Our optimised VAMS method increased protein IDs by a smaller margin than plasma, with a 2-fold increase detected. However, the protein ID numbers from the liquid samples were overall higher than plasma. %CVs were reduced from 14.2% to 8.5%, a larger improvement than for plasma.

PLASMA

2. Effect of processing steps on plasma results

Figure 2.1: Number of proteins identified in plasma and serum samples (n=3).

Plasma and serum was produced using different anticoagulants and then applied to VAMS tips. Protein numbers detected in technical replicates was highly variable with the exception of EDTA and heparin plasma. ID numbers varied from 872 at the lowest (EDTA) to 1659 at the highest (citrate). This was a variation of 90%.

Figure 2.1

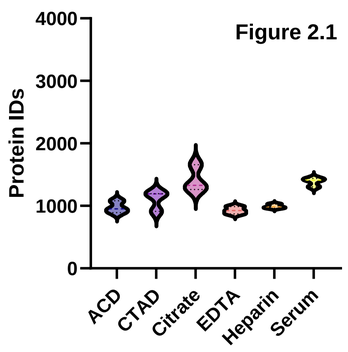


Figure 2.2: Number of proteins identified in plasma centrifuged once or twice and overlap of detected proteins.

EDTA plasma was produced by either single spinning (ss) or double spinning (ds) plasma – both standard techniques for plasma production. Double spinning plasma reduced protein IDs by 53% and of those, 96% overlapped with single spun samples.

Figure 2.3: Number of proteins identified in plasma produced with 2 different centrifuges.

EDTA plasma was produced by centrifuging EDTA whole blood in two different benchtop centrifuges at 1500*g* for 10 minutes. Despite being subjected to the same settings, the differences in detected protein IDs was 2.5-fold and highly significant.

Figure 2.2

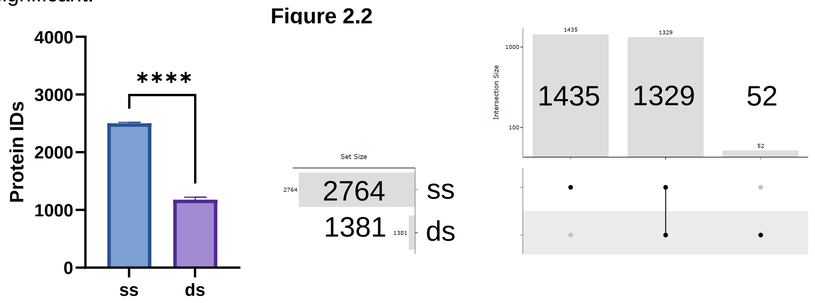
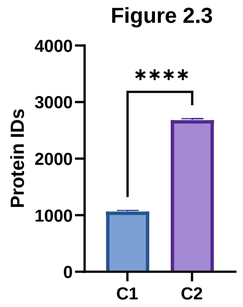


Figure 2.3



WHOLE BLOOD

3. Whole blood outperforms even the best plasma method

Figure 3.1

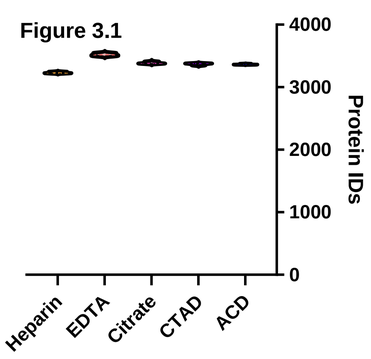


Figure 3.1: Number of proteins identified in whole blood samples with different anticoagulants (n=3).

Whole blood was collected into vacutainers with different anticoagulants and then applied to VAMS tips. Protein numbers detected in technical replicates were highly reproducible for every anticoagulant. ID numbers varied from 3220 at the lowest (heparin) to 3546 at the highest (EDTA). This was a variation of 10.1%.

Figure 3.2: Dynamic range of detected proteins is reduced in whole blood.

Dynamic range of whole blood (grey) was attenuated compared to single spun plasma (yellow) and double-spun plasma (blue).

Figure 3.3: Whole blood from VAMS produces more protein and peptide IDs compared to plasma (24% higher in whole blood).

Figure 3.4: Whole blood has a higher reproducibility compared to plasma.

(WB CV: 6.1%; ssPlasma CV: 10.7%; dsPlasma CV: 14.8%)

Figure 3.2

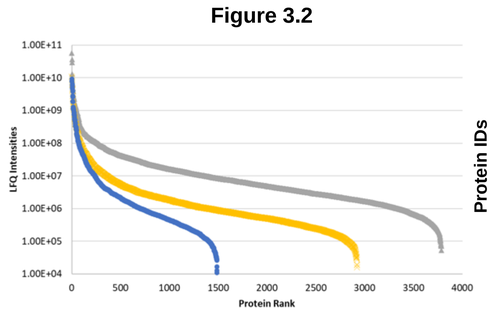


Figure 3.3

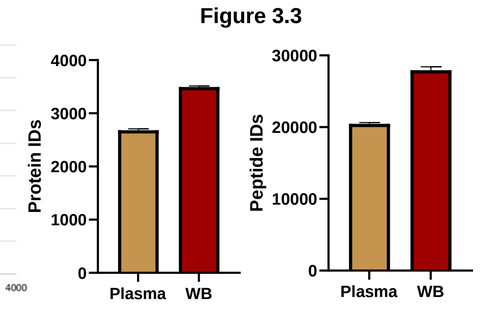
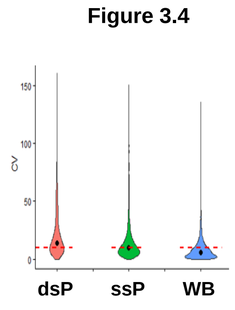


Figure 3.4



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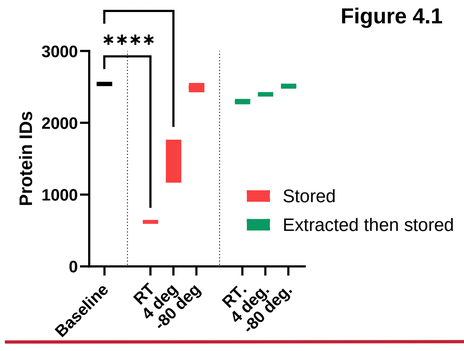
PLASMA

4. Effect of long-term storage

Figure 4.1



Plasma and whole blood VAMS were prepared. A subset were processed and analysed immediately (baseline), and the remainder were stored for 3 months with desiccant at room temperature (RT), 4°C (4deg), or -80°C (-80deg). Of the stored samples, half were washed according to the optimised VAMS protocol before storing and the other half were washed after storage. Following this, all samples were digested and analysed.

Figure 4.1: Number of proteins identified in plasma after long-term storage.

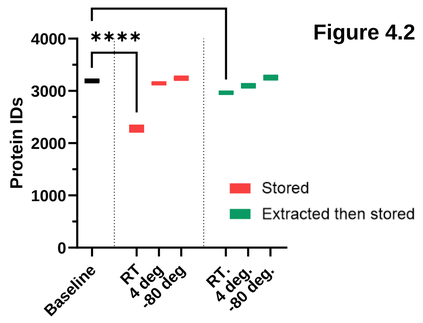
Plasma samples were highly affected by storage temperature with a 76% reduction in protein IDs after storage at room temperature and 42% reduction at 4°C.

Figure 4.2: Number of proteins identified in whole blood after long term storage.

Whole blood only lost 29% of IDs after room temperature storage and no detectable loss at 4°C or colder.

WHOLE BLOOD

Figure 4.2



CONCLUSIONS

1. **Collection of any blood sample (plasma, serum, or whole blood) into a VAMS device before extraction improves sensitivity and increases the number of identified proteins by 2 - 3.5-fold.**
2. **Whole blood samples stored for 3 months were more stable than plasma samples and were less effected by storage temperature. The optimal storage temperature for all samples in collected in VAMS was -80°C.**
3. **When immobilised in VAMS, whole blood is more reproducible, produces more datapoints, and is more resilient to temperature associated changes than plasma.**

Acknowledgements

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References

1. Molloy MP, Hill C, O'Rourke MB, Chandra J, Steffen P, McKay MJ, Pascovici D, Herbert BR. Proteomic Analysis of Whole Blood Using Volumetric Absorptive Microsampling for Precision Medicine Biomarker Studies. J Proteome Res. 2022 Apr 1;21(4):1196-1203. doi: 10.1021/acs.jproteome.1c00971. Epub 2022 Feb 15. PMID: 35166117.

Sangui Bio

Funding sources

1. Sangui Bio Pty Ltd

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