# Nanoparticle-assisted proteomics of whole blood collected through multiple dried blood spot collection devices

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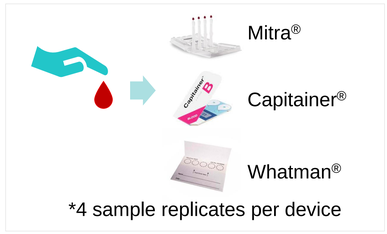
## The Proteograph™ Product Suite enables reproducible and deep proteomic analysis from whole blood microsampling devices

### Introduction

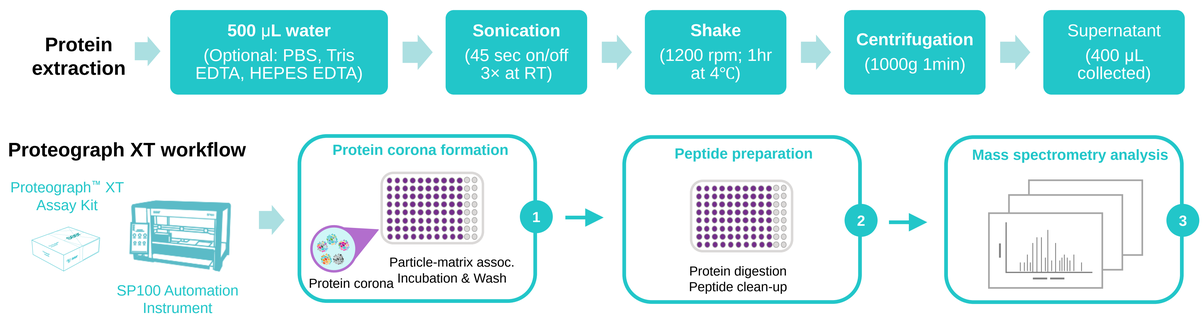
Microsampling is a methodology for collection and analysis of low-volume biological samples, typically under 50 µL. It affords several advantages compared to conventional sampling methods: diminished invasiveness, enhanced convenience, and cost-effectiveness. It allows collecting various biological specimens from diverse sources, finding widespread application in clinical trials, therapeutic drug monitoring, pharmacogenomics and disease diagnosis.

However, similar to plasma, microsampling of whole blood has a huge dynamic range issue as it has all the components of plasma along with heme and cells. Although in some cases the low volume is beneficial, it also adds the challenge of sensitivity for a number of assays and quantification.

In this study, we evaluate the performance of 3 microsampling devices and show that combining microsampling with nanoparticle-based Seer Proteograph™ XT workflow significantly improves proteome coverage. Capillary blood were collected from two participants via finger-prick, using 30 µL Mitra™ devices, 10 µL Capitainer™ device, and Whatman™ paper. Simultaneously, intravenous blood draw was obtained from the same participant and allocated to Mitra devices.




### Evaluation of 3 microsampling devices with the Proteograph XT workflow

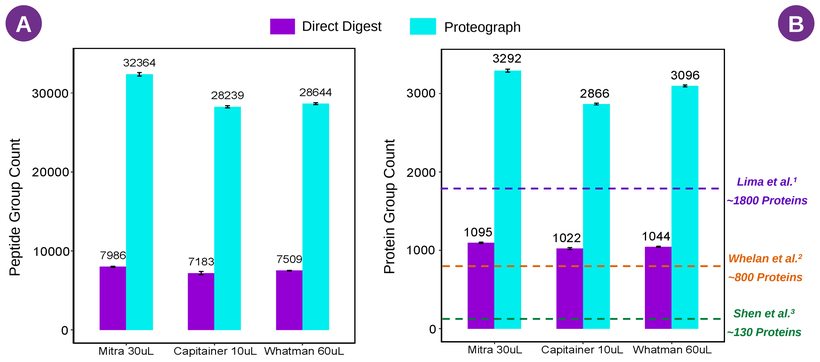


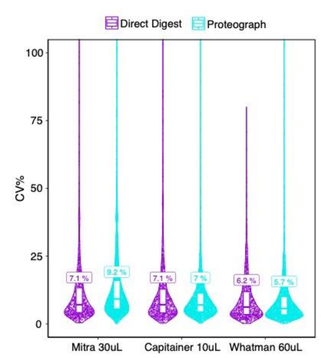

**Data analysis.** Proteomics data was collected in DIA using a 45-minute gradient on Orbitrap Exploris™ 480 and analyzed with DIA-NN v1.8.1 in library free mode, with MBR. Peptides were filtered out with *q*-value<0.01, normalized using global median normalization and rolled up to protein groups using maxRep method.

## Proteograph™ XT workflow provides unbiased proteomics analyses from whole blood microsampling devices

### Proteograph XT workflow significantly enhances proteome coverage while maintaining high reproducibility

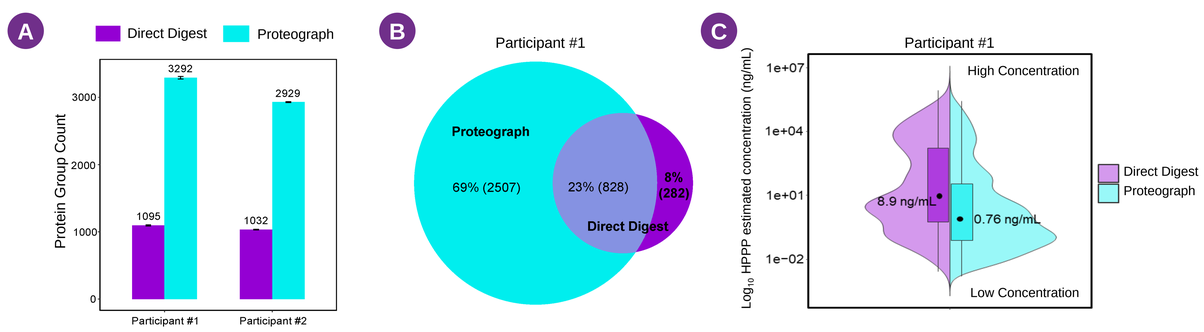
**(A)** Proteograph with nanoparticles enables approximately 30,000 peptides and 3,000 protein groups quantified in each of the microsampling devices, deeper than three published references and **(B)** consistently manifest low coefficients of variation (CVs) of protein groups below 10% across 4 process replicates.



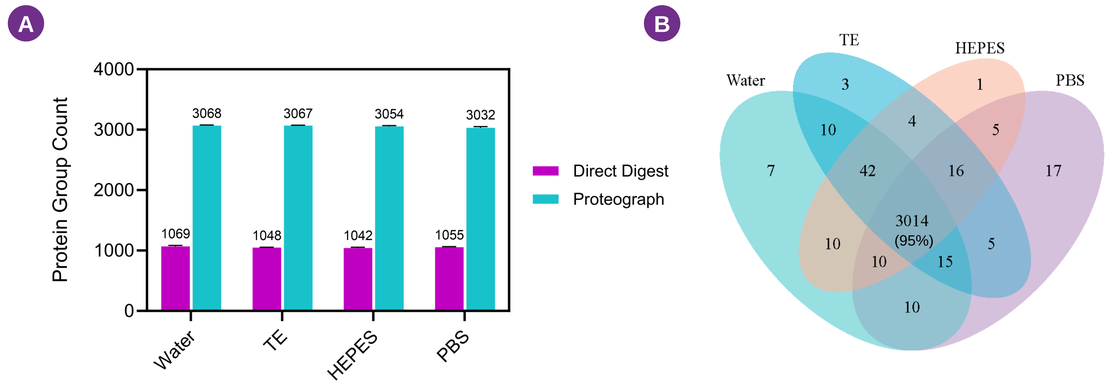

### Proteograph XT workflow enables measurement of low abundant proteins

**(A)** Approximately 3,000 proteins were consistently quantified in Mitra + Proteograph XT workflow across two participants, boosting 3 times more proteins than direct digestion. **(B)** Mitra + Proteograph captured 92% of the proteins quantified from Proteograph and direct digest. **(C)** Proteograph yielded more low concentration proteins with median concentration at 0.76 ng/mL compared to 8.9 ng/mL of direct digestion based on HPPP database[4].



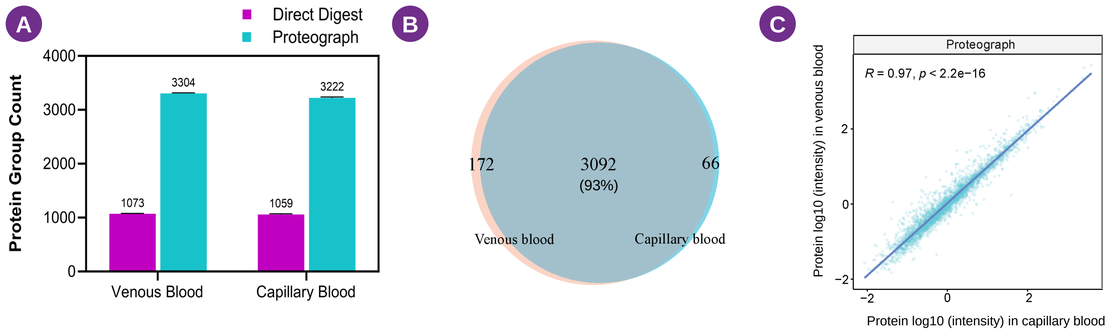

### Proteograph XT workflow is compatible with various protein extraction buffers

**(A)** More than 3000 protein groups were consistently quantified in Mitra + Proteograph XT workflow using different extraction buffers (water, PBS, TE (10 mM Tris + 1 mM EDTA) and HEPES (20 mM HEPES + 1 mM EDTA) and **(B)** showed comparable protein groups with an overlap of 3014 (95%) proteins across all five tested buffers.




### Capillary blood achieves comparable results to venous blood

**(A)** Around 3,200 protein groups were captured in capillary blood using Mitra + Proteograph XT workflow and **(B)** showed a high overlap (93%) and **(C)** intensity correlation with the ones from venous blood using Proteograph.




## Conclusion

- Proteograph XT workflow enables deep protein coverage (~3,000 proteins) in whole-blood collected through microsampling devices, comparable to venous blood.
- Consistent high protein coverage was observed in two participants and multiple different extraction buffers.
- The capability for unbiased proteomic exploration at such an unprecedented depth with microsampling combined with the Proteograph XT workflow, opens new opportunities for low-volume blood proteomics including the early discovery of biomarkers and the non-invasive monitoring of health status.

## References

[1] Lima *et al., J. Proteome Res. (2022) DOI: 10.1021/acs.jproteome.1c00892*
[2] Whelan *et al., Anal. Chem.* (2023) *DOI: 10.1021/acs.analchem.3c01189*
[3] Shen *et al., Nat. Biomed. Eng.* (2023) *DOI: 10.1038/s41551-022-00999-8*
[4] Schwenk *et al., J. Proteome Res. (2017) DOI: 10.1021/acs.jproteome.7b00467*

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