

# HAEMOGLOBIN INTERFERENCE IN DRIED BLOOD SPOT PROTEOMICS FOR CHRONIC KIDNEY DISEASE



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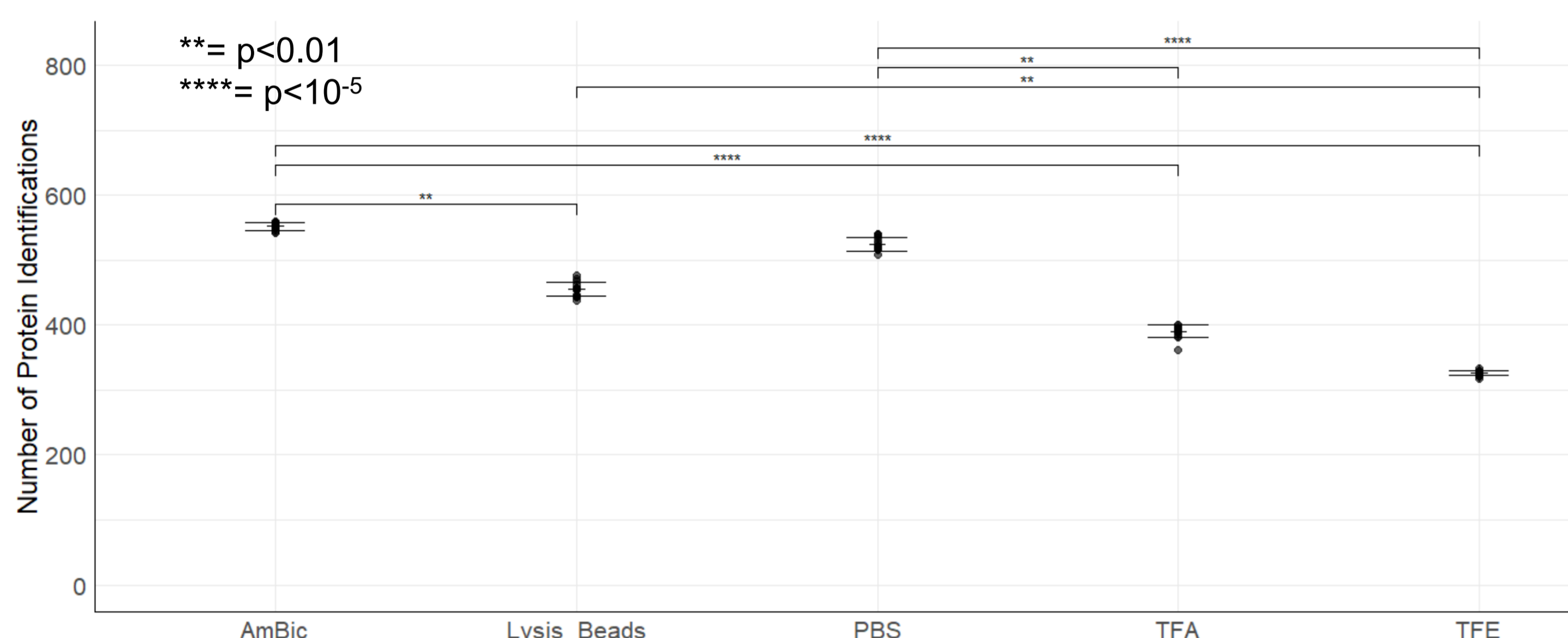


## INTRODUCTION

The use of **dried blood spots** is an exciting prospect for longitudinal and at home sampling of **paediatric kidney patients**. This has the potential to avoid children missing school and reduce costs associated with attending medical appointments, as well as promoting the **Save the Vein** initiative, whilst providing high quality samples for **biomarker discovery** and **screening**. It may also allow for participation of underrepresented populations in clinical research due to the ability for **remote sampling**. Proteomics allows characterisation of the **blood proteome** using **mass spectrometry**. However, the presence of high abundance proteins, such as **haemoglobin**, can mask low abundance proteins making biomarker discovery less likely. By establishing methods to **eliminate haemoglobin** from reconstituted dried blood spots, other key proteins can be identified in Chronic Kidney Disease (CKD) patients.

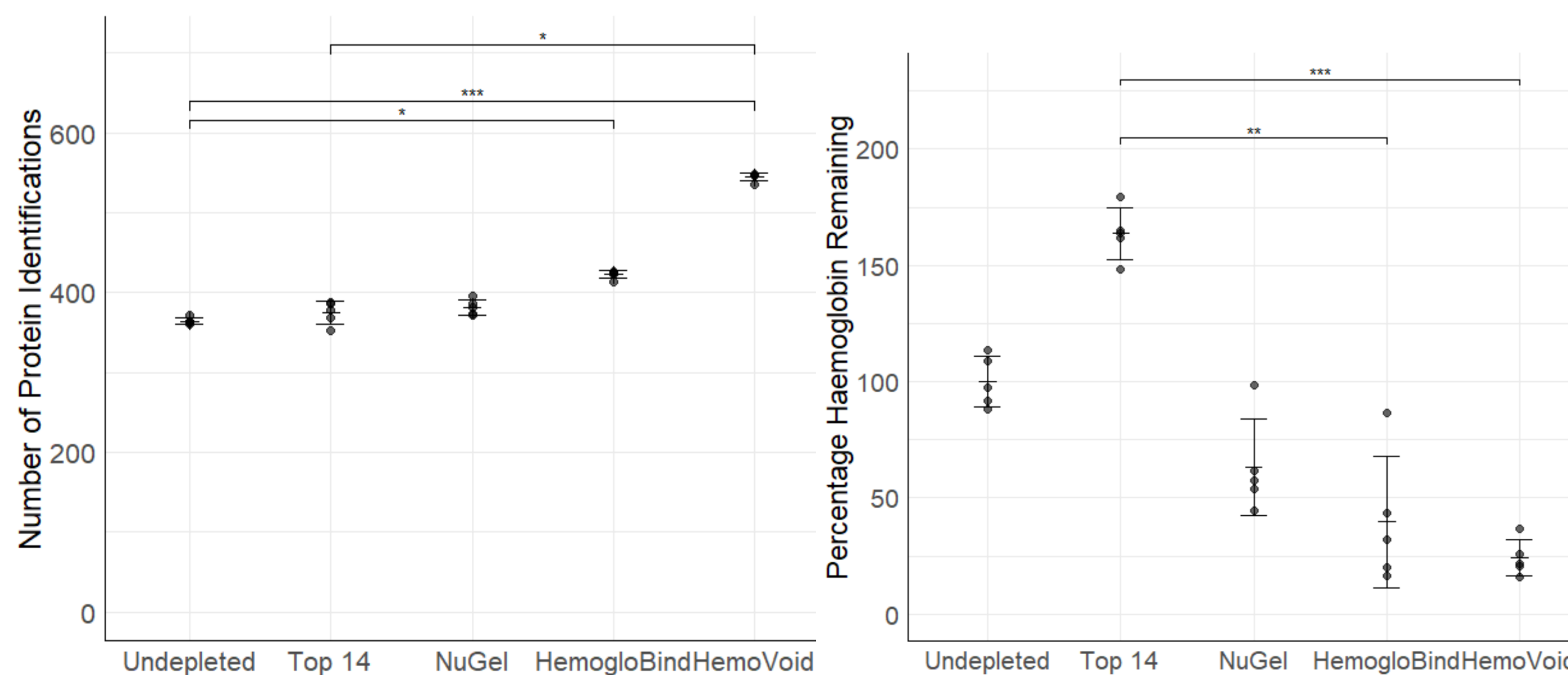
## RESULTS

### Optimisation of dried blood spot proteomics



Incubation of the 10  $\mu$ L dried blood spot with 200  $\mu$ L ammonium bicarbonate for 2 hours, shaking at 800 rpm gave the highest number of protein identifications with the lowest variation (RSD= 1.28%). No significant difference was identified in protein identifications across the three sample collection devices evaluated (Capitainer b10, VAMs Mitra and Whatman903) ( $p>0.999$  for all). Capitainer devices were used for the rest of the workflow due to their reported increased ease of use.

\*=  $p<0.05$   
\*\*\*=  $p<0.001$

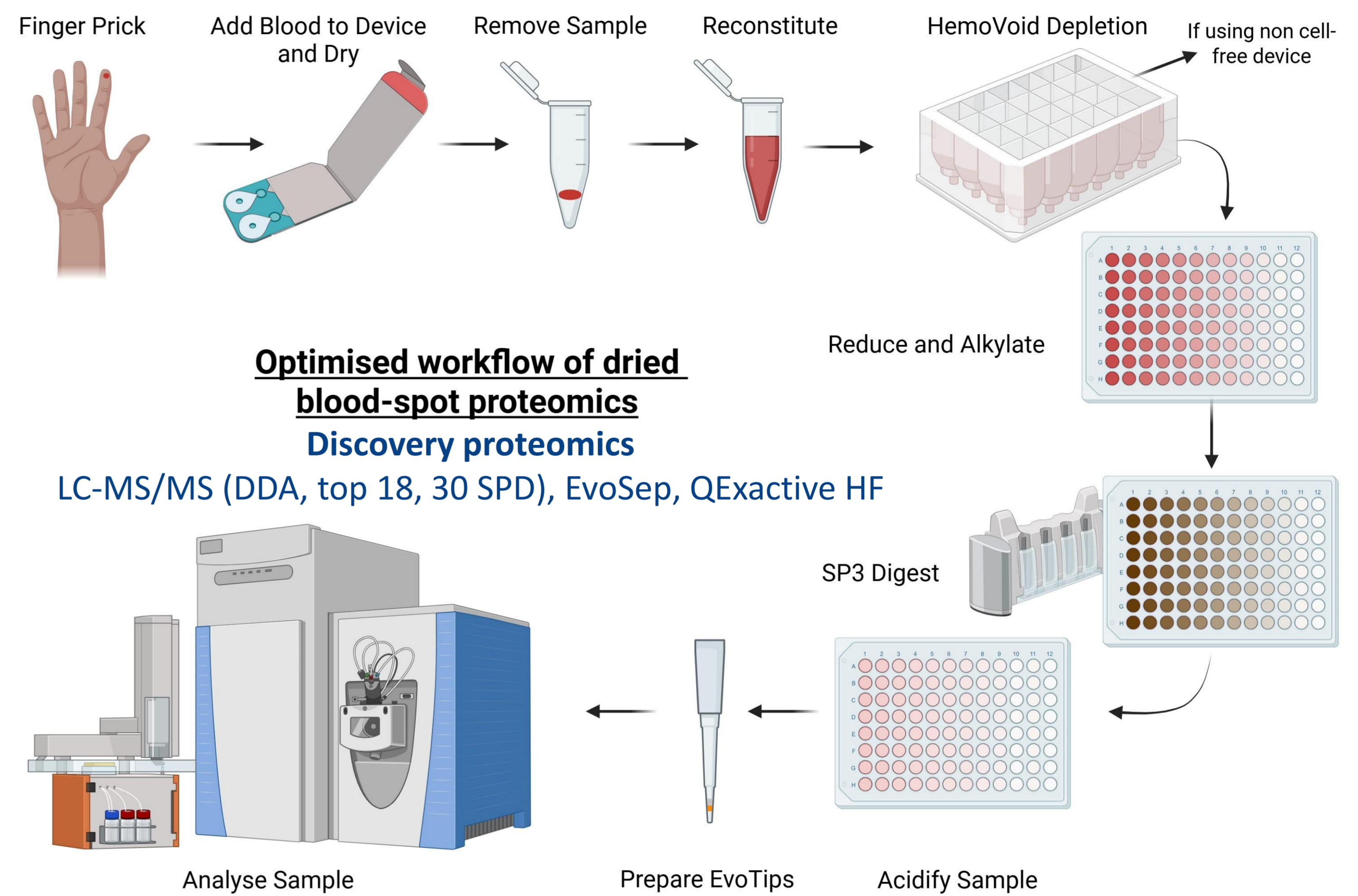


Comparison of three haemoglobin depletion kits (Biotech Support Group), along with Top 14 (ThermoFisher), found that HemoVoid removed the highest proportion of haemoglobin ( $75.79\% \pm 7.9\%$ ), with the lowest level of variation, resulting in the highest number of identified proteins ( $545 \pm 5$ ).

Annesley, T.M. (2003) Ion Suppression in Mass Spectrometry. *Clinical Chemistry*, 49 (7), pp.1041-1044.  
Marsh AM, Genova R, Buicko Lopez JL. Dialysis Fistula. [Updated 2023 May 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK559085/>  
Rosting, C., Yu, J. & Cooper, H.J. (2018) 'High Field Asymmetric Waveform Ion Mobility Spectrometry in Nontargeted Bottom-up Proteomics of Dried Blood Spots', *Journal of Proteome Research*, vol. 17, no. 6, pp. 1997-2004.  
Shuken, S.R. (2023) 'An Introduction to Mass Spectrometry-Based Proteomics', *Journal of Proteome Research*, vol. 22, no. 7, pp. 2151-2171.  
Velghe, S. & Stove, C.P. (2018) Evaluation of the Capitainer-B Microfluidic Device as a New Hematocrit-Independent Alternative for Dried Blood Spot Collection. *Analytical Chemistry*, 90 (21), pp.12893-12899. Available from: <https://dx.doi.org/10.1021/acs.analchem.8b03512>.

## MATERIALS AND METHODS

### Developed workflow for dried blood spot proteomics

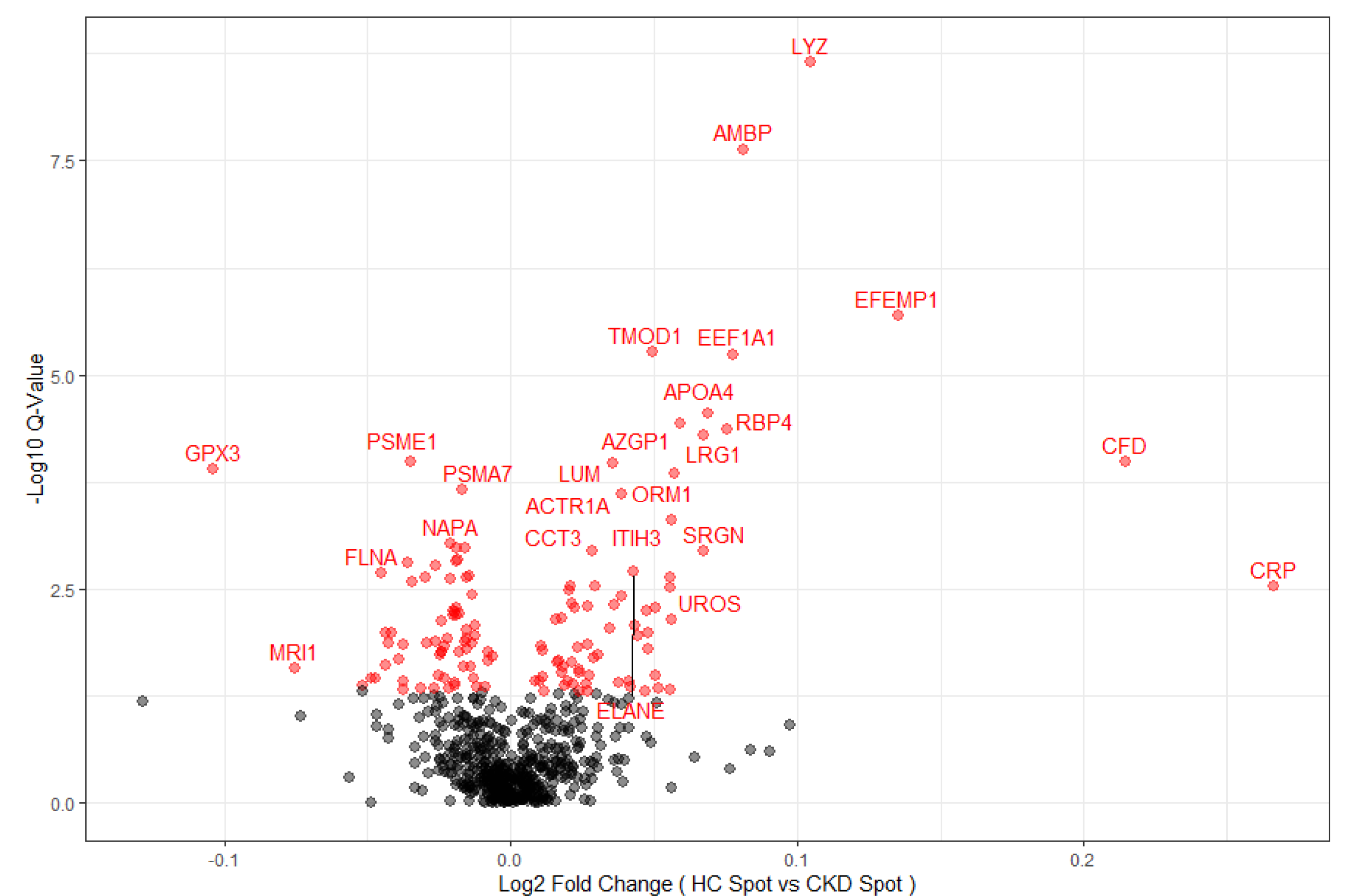


### Optimised workflow of dried blood-spot proteomics

#### Discovery proteomics

LC-MS/MS (DDA, top 18, 30 SPD), EvoSep, QExactive HF

### Translation to clinical samples



When this method was applied to dried blood spots from Stage V CKD patients on dialysis for proof of principle (eGFR median  $5.50 \pm 2$  mL/min/1.73 m<sup>2</sup>) (GlomOmics, REC 23/PR/0490), 136 proteins were identified as being statistically different compared to the healthy controls. The largest significant difference was observed in C Reactive Protein (CRP) ( $Q= 2.9 \times 10^{-3}$ ,  $\text{Log}_2\text{FC}= 0.27$ ), which was significantly higher in the CKD group than the healthy controls ( $n= 10$ ). This method allowed for the identification of potential clinically relevant biomarkers in CKD patients receiving haemodialysis, using a starting volume of just 10  $\mu$ L of blood, and avoiding additional venepuncture.

## CONCLUSIONS

**Resuspension in 50 mM Ammonium Bicarbonate for 2 hours at 800 rpm resulted in the highest number of protein identifications**

**The quantity of haemoglobin removed from the sample correlated with the number of proteins identified, and HemoVoid removed the most haemoglobin**

**This method allowed for the identification of potential clinically relevant biomarkers in CKD patients receiving haemodialysis**

**This demonstrates a role for remote sampling in longitudinal studies, and frequent testing; supporting the save the vein initiative**