PREOMICS

ENRICHplus enables enhanced plasma proteome coverage while preserving quantitative accuracy

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INTRODUCTION

Blood is a rich source for biomarker discovery, containing a wide proteome of cytokines and tissue leakage proteins linked to disease. However, MS-based plasma proteomics faces challenges due to the broad dynamic range of protein concentrations and the suppression of low-abundance proteins by high-abundance proteins, which can hinder their detection. Enrichment methods can improve coverage but often distort protein ratios, making accurate quantification difficult. ENRICHplus, a bead-based sample preparation method, addresses these issues by compressing the dynamic range while preserving quantitative accuracy. A Controlled Quantitative Experiment (CQE) was performed to compare ENRICHplus with a neat plasma workflow in terms of coverage, reproducibility, and accuracy.

MATERIALS

Material: K2EDTA pooled bovine plasma (NeoBiotech, France) was spiked into a K2EDTA pooled human plasma sample in quadruplicates with the following vol/vol ratios: 1:0, 9:1, 1:1, 1:9, 0:1 (Bovine:Human).

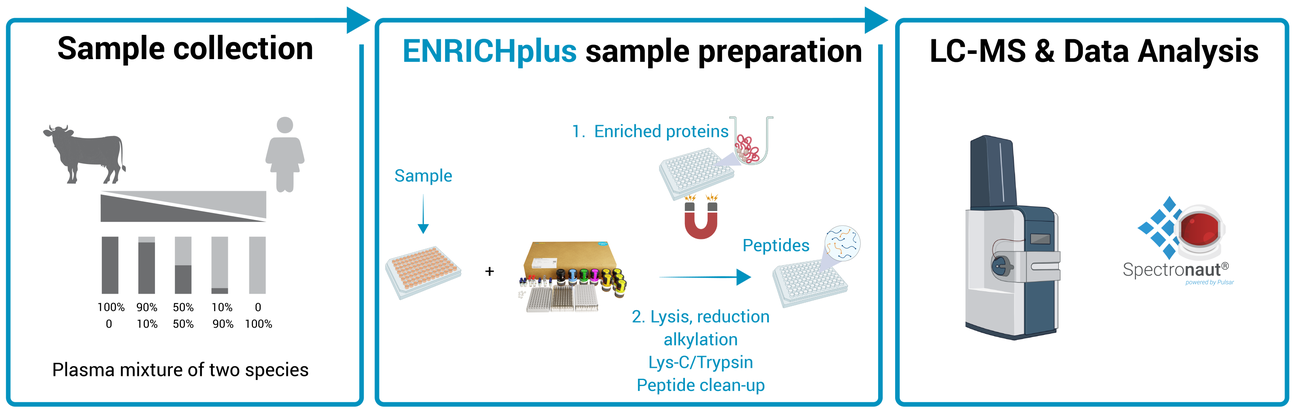
METHODS

Sample preparation: For ENRICHplus samples, 50 µL of plasma were processed with the ENRICHplus workflow. For neat samples, 2 µL of plasma were prepared following the iST-BCT protocol.

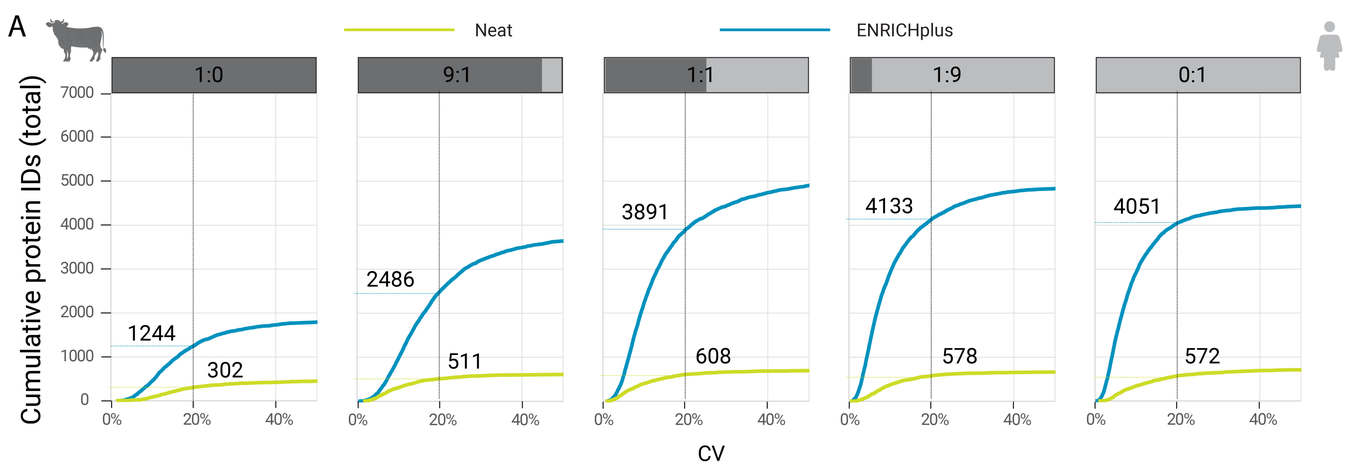
LC-MS analysis: 300 ng of peptides were analyzed using an nanoElute®2 (Bruker) equipped with an Aurora™ Ultimate CSI 25×75 C18 UHPLC column (IonOpticks) using a 30-minute gradient and coupled to a timsTOF HT (Bruker) in dia-PASEF® mode.

Data processing: Spectronaut® 19 using directDIA+™ mode.

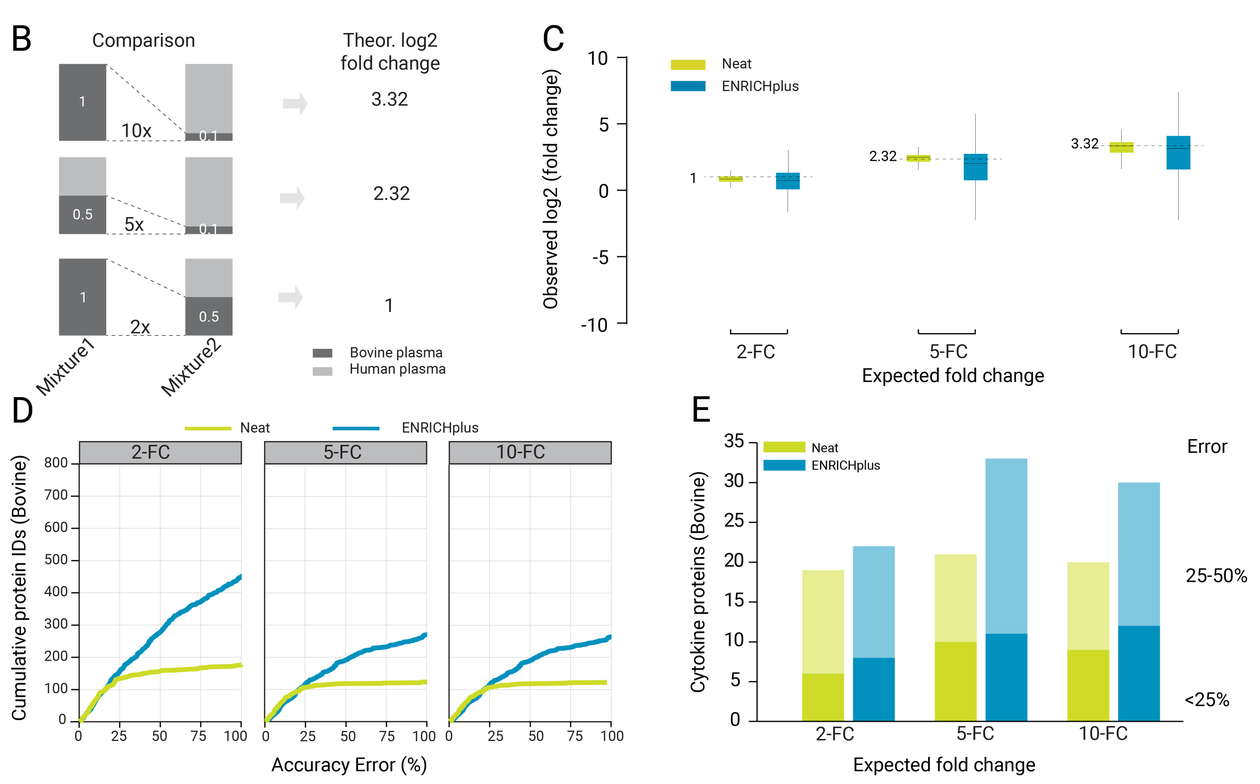
RESULTS



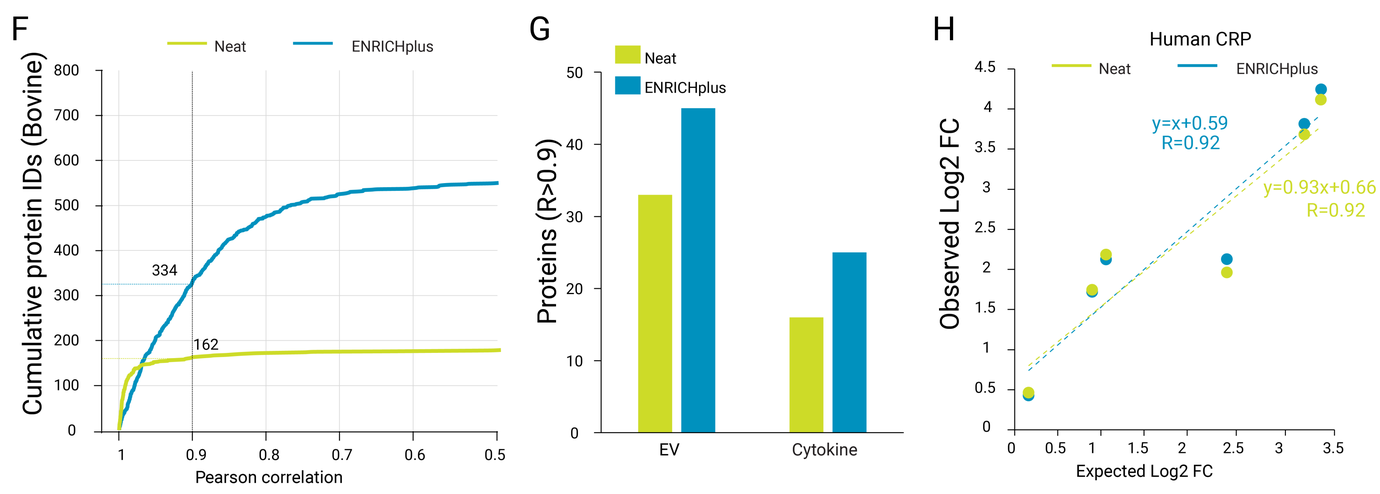
Workflow overview for plasma preparation using ENRICHplus.



Enhanced proteome coverage and precision by ENRICHplus in CQE study. A) ENRICHplus compressed the dynamic range of the plasma proteome, increasing the number of precisely quantified proteins (CV <20%) by 4- to 7-fold compared to neat plasma, depending on the mixing ratio and species. Quantification precision improved with increasing amounts of human plasma, highlighting the method's specificity for human samples.



High accuracy demonstrated by ENRICHplus. B) Quantified proteins from two different mixtures were combined to calculate the expected fold change. C) Both ENRICHplus and neat plasma samples demonstrated high accuracy and strong comparability. D) ENRICHplus and neat plasma showed comparable performance within the high-accuracy error range (<25%). E) Within the acceptable error range (<50%), ENRICHplus quantified nearly twice as many proteins as neat samples, including a greater number of low-abundance proteins such as cytokines.



High linearity achieved with ENRICHplus. F) Proteins quantified by both ENRICHplus and neat plasma showed a strong linear correlation. G) With ENRICHplus, more EV- and cytokine-related proteins were quantified among those with a Pearson correlation coefficient >0.9. H) For the highly expressed human inflammatory protein CRP, ENRICHplus provided comparable high accuracy while maintaining a strong linear relationship across different fold changes.

KEY TAKEAWAYS

- **Enhanced proteome coverage**
  ENRICHplus significantly increases the number of quantified proteins, particularly low-abundance targets like cytokines, by compressing the plasma proteome's dynamic range.
- **Improved quantitative precision**
  Up to 7-fold more proteins were quantified with high precision (CV <20%) compared to neat plasma, with specificity for human plasma samples.
- **High quantitative accuracy**
  ENRICHplus maintains strong comparability to neat plasma workflows, with a similar number of proteins showing <25% error and nearly twice as many protein quantifications within an acceptable error range (<50%).
- **Strong linearity and biological relevance**
  ENRICHplus preserves linearity in quantification across mixtures and enhances the detection of biologically relevant proteins, including EV- and inflammation-related markers like CRP.

CONTACT & MORE

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Conflict of Interest Disclosure
Moritz, C., Hu, Z., Limm, K., Wurzenberger, F., Boateng, G., and Kulak, N. are employed by PreOmics GmbH.