Automation of Nanoparticle-Based Plasma Enrichment on the Opentrons Platform Enables Scalable, Reproducible Plasma Proteomics

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Introduction

Mass spectrometry-based plasma proteomics holds strong potential for biomarker discovery but remains challenged by limited proteome depth and the need for highly reproducible sample preparation across large cohorts. Nanoparticle-based enrichment workflows improve access to low-abundance plasma proteins; however, their broader adoption depends on robust, scalable automation. This study evaluates semi-automation of the *P2-iST Plasma* workflow on the Opentrons liquid-handling platform, benchmarked against manual processing. As bead handling represents the primary source of variability in enrichment-based workflows, its automation enables improved control and consistency, while downstream processing remains inherently robust. In parallel, we investigate chromatographic optimization strategies—specifically gradient length and column configuration—to further extend plasma proteome depth within a scalable LC–MS/MS workflow.

Materials and Methods

Experimental Design

To investigate the impact of controlled bead handling on proteome depth and reproducibility, human plasma samples were processed using the *P2-iST Plasma* workflow under two conditions: fully manual preparation and semi-automated enrichment.

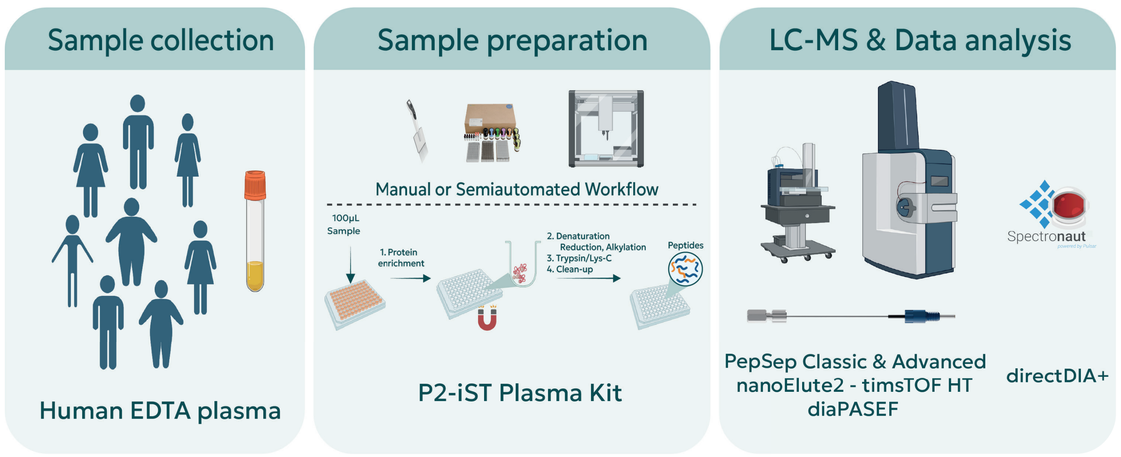

Figure 1. *P2-iST Plasma* workflow.

Sample Preparation, LC-MS and Data Analysis

Human EDTA plasma samples (100 µL; n = 20 healthy donors; BioIVT) were processed using the *P2-iST Plasma* workflow (Biognosys Group), either manually or with semi-automated enrichment on the Opentrons OT-2 platform. The workflow combines single nanoparticle-based enrichment with standardized downstream processing (lysis, reduction, alkylation, digestion, and peptide cleanup using *iST* chemistry; PreOmics) in a 96-well format. In the semi-automated workflow, bead handling was performed on the Opentrons platform, while all downstream steps remained identical. Technical replicates and pooled QC samples were included to assess reproducibility and workflow consistency. For neat plasma preparation, 2 µL sample was processed using *iST-BCT* workflow (PreOmics) according to instructions. Peptides (600 ng) were analyzed by nanoLC–MS/MS (*timsTOF HT, diaPASEF*) using *PepSep Classic* (48SPD) and *Advanced* (24SPD) columns. Data were processed with *Spectronaut 21* (*directDIA+*). Performance was assessed by protein identifications, data completeness, coefficient of variation (CV).

Results

Controlling Bead Handling Improves Depth

Using pooled QC plasma samples, *P2-iST Plasma* increased protein identifications by ~6-fold compared to neat plasma (~5,300-5,600 vs. ~900 protein groups) while reducing quantitative variability. Similar performance between manual and OT-2 workflows demonstrates that controlled bead handling during enrichment markedly improves analytical depth without compromising reproducibility (Fig. 2).

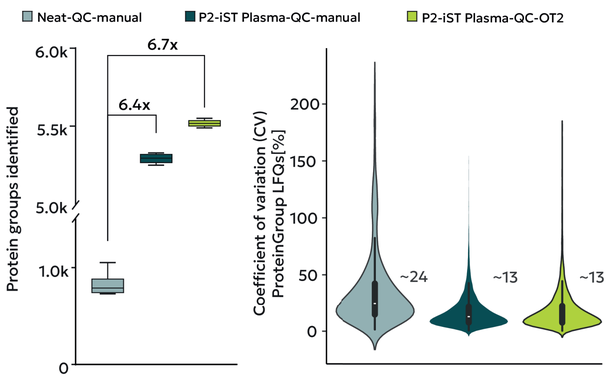

Figure 2. Protein identifications and variability in pooled QC plasma samples. Left: Protein group identifications for neat plasma and *P2-iST Plasma* workflows. *P2-iST Plasma* resulted ~5,300-5,600 protein groups compared to ~900 for neat plasma. **Right:** Distribution of protein group coefficients of variation (CVs) across workflows. *P2-iST Plasma* samples showed lower median CVs (~13-15%) than neat plasma (~24%).

Conflict of Interest Disclosure: K.Y., A.B., Z.H., S.W., and K.L. are employed by PreOmics GmbH; S.D. is employed by biocrates life science GmbH; K.W. and B.L. are employed by Opentrons Labworks Inc.

Robust Performance Across the Cohort

Across 20 donor plasma samples, *P2-iST Plasma* consistently identified ~20k-40k peptides and ~3k-5k protein groups per sample despite substantial biological variability. Comparable performance between manual and OT-2 preparations demonstrates the robustness and scalability, while minimal signal in blanks confirms low background and negligible cross-contamination, supporting large-cohort analysis (Fig. 3).

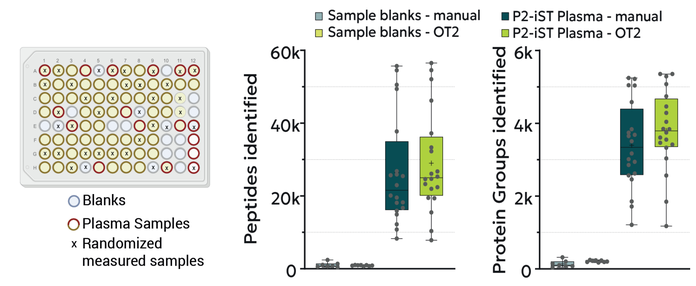

Figure 3. Cohort-wide workflow performance. Left: Randomized 96-well plate layout containing donor plasma samples, pooled QC plasma samples, and blanks. **Right:** Peptide and protein group identifications across 20 donor samples processed using manual and OT-2 *P2-iST Plasma* workflows. Blank preparations served as negative controls.

P2-iST Plasma delivered highly consistent protein group identification across manual and OT-2 workflows, with 4,407 shared proteins detected between preparations. Compared to neat plasma (733 proteins), enrichment increased proteome coverage to >5,200 protein groups and reduced the dominance of high-abundance proteins (~59% to ~13-16% intensity fraction). These results demonstrate improved access to previously underrepresented components (Fig. 4).

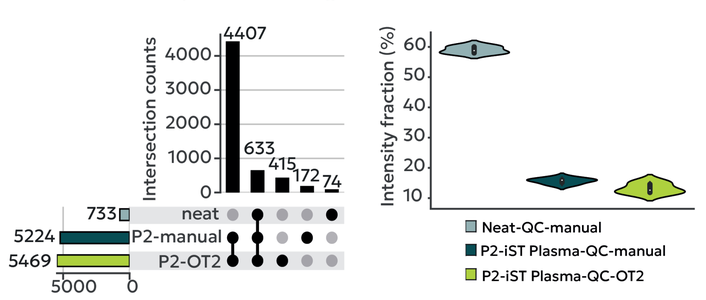

Figure 4. Protein overlap and intensity distribution across workflows. Left: UpSet plot showing protein group overlap between workflows using pooled QC plasma samples. **Right:** Distribution of relative protein intensity fractions (%) across workflows, illustrating differences in signal allocation across the plasma proteome.

Improved Access to Low-Abundance and Clinically Relevant Proteins

P2-iST Plasma substantially expanded proteome coverage across the full abundance range, increasing detectable proteins from ~700 in neat plasma to >5,000 with enrichment and >6,000 under optimized chromatographic conditions. While high-abundance proteins remained consistently detected, enrichment extended protein identification into lower-abundance regions of the plasma proteome. This increased depth enhanced detection of biologically and clinically relevant protein classes, including cytokines, CD-related proteins, and FDA-listed biomarkers, with further gains achieved using the *PepSep Advanced* column (Fig. 5).

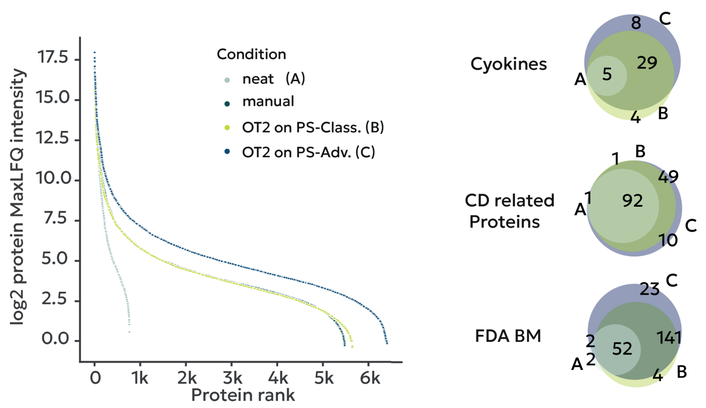

Figure 5. Expanded dynamic range enables detection of biologically relevant proteins. Left: Rank-ordered protein intensities (log2 MaxLFQ) for neat plasma and *P2-iST Plasma* workflows using manual and OT-2 preparation with *PepSep Classic* and *Advanced* columns. **Right:** Overlap analysis of cytokines, CD-related proteins, and FDA-listed biomarkers detected in neat plasma (A), *P2-iST Plasma* OT-2 with *PepSep Classic* (B), and *PepSep Advanced* (C).

Key Takeaways

- **Deeper plasma proteome:** *P2-iST Plasma* enables ~6× increased plasma proteome coverage compared with neat plasma.
- **Enhanced performance through partial automation:** Controlled bead handling reduces variability, improves consistency, and supports scalable high-throughput workflows.
- ***PepSep* UHPLC columns deliver high-performance separations:** *Classic* enables high-throughput workflows, while *Advanced* column provides maximum plasma proteome depth.

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