Proof-of-concept study: A cost-efficient and scalable plasma proteomics pipeline utilizing the Evotip Pure subjected to more than 1,100 patient samples

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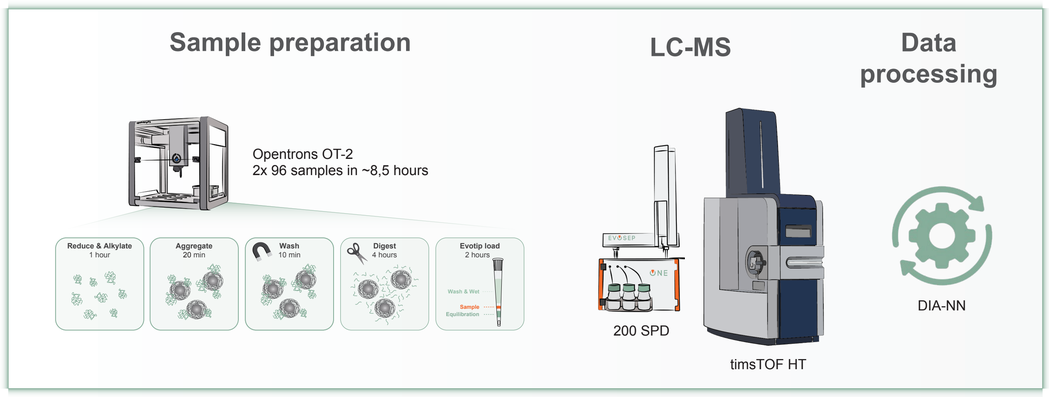
Highlights

- Complete, Evotip Pure-based workflow solution for cost-efficient and reproducible large-scale plasma proteomics.
- Ultra-low carryover (<0.2%) across 1,100+ plasma samples with the Evotip Pure and the 200 SPD method on the Evosep One.
- Unmatched stability and reliability of Evosep One ensures consistent performance across patient cohort collected from two hospital sites.

Scalable workflow powered by Evotip Pure

Optimized for large-scale plasma proteomics

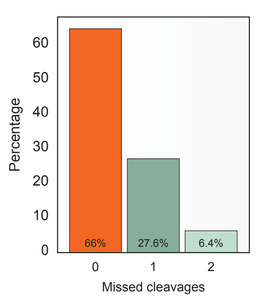
Plasma proteomics advances biomarker discovery but scaling challenges efficiency, cost, and reproducibility. We describe a cost-efficient, automated workflow leveraging the Opentrons OT-2 and Evotip Pure technology for miniaturized sample preparation of plasma (1 µL). Combined with high-throughput LC-MS it processes 1,100+ samples in under seven days showcasing the advantages of Evotip Pure and Evosep One.



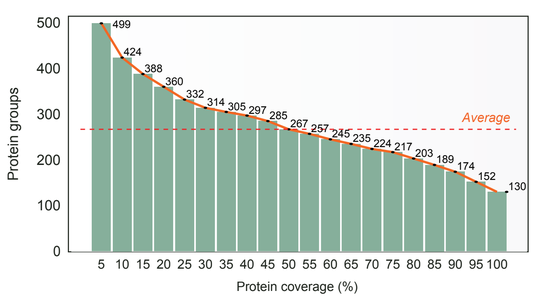
Schematic representation of the fully automated digestion workflow for 1 to 192 samples, and overall analytical workflow

Analysis of 1,122 patient plasma samples

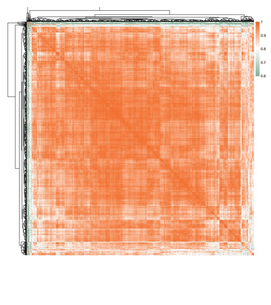
Our workflow enabled high-throughput LC-MS analysis of 1,122 patient plasma samples with excellent performance. A four-hour ambient digestion with trypsin and Lys-C ensured high cleavage efficiency, achieving robust proteome coverage. On average, 267 proteins were detected per sample, with 200 proteins consistently identified in 80% of patient samples.



Digestion efficiency, precursur level (4h, ambient temperature).



Data completeness assessment on protein level across all patient plasma samples (n = 1,122).

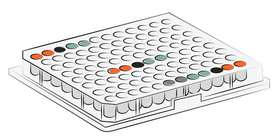


Sample-to-sample Pearson correlation of all patient samples from 15 plates.

Seamless integration with outstanding performance

Reliable and robust workflow solution

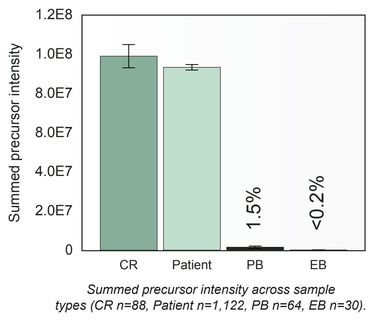
To evaluate workflow performance, we systematically assessed key metrics across the patient cohort, incorporating diverse QC samples for real-time monitoring of robotic precision, LC-MS stability, and data reproducibility.



	Samples	*Description*
Evotip Blank (EB)	30	Solvent A loaded Evotip Pure to check for LC carryover.
System Suitability Control (SSC)	88	50 ng HeLa loaded on Evotips to check system suitability.
Plate Blank (PB)	64	Processed blank with all reagents except sample to monitor cross-well contamination.
Common plasma reference (CR)	88	Pool of all patient samples, run across each plate for inter-plate variability.
Patient plasma sample	1,122	15 x 96-well plates analyzed, containing up to 79 patient samples.
Total # samples	**1,392**	

Evotip Blank (EB)

Evotip blanks (EB) across the entire experiment of more than 1,200 patient and CR samples confirmed <0.2% carryover by the Evosep One system.



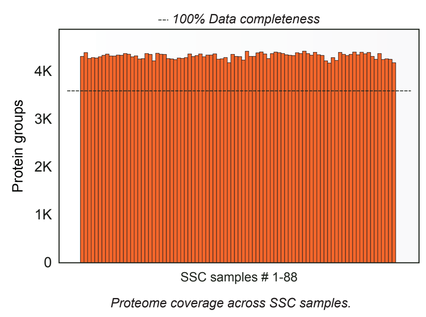
Summed precursor intensity across sample types (CR n=88, Patient n=1,122, PB n=64, EB n=30).

Plate Blank (PB)

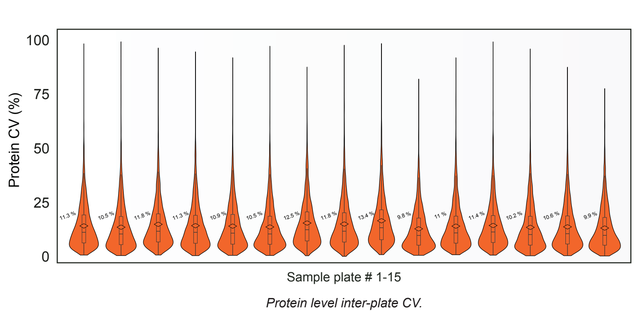
Consistent plasma intensities (patient & CR) and negligible PB signals confirm no cross-well contamination, positional bias, or carryover in the complete workflow. Any detectable PB signal arises from workflow enzymes and reagents.

System Suitability Control (SSC)

Incorporating 50 ng HeLa samples (SSC) enabled consistent LC-MS performance monitoring, ensuring excellent proteome coverage and precision (12% median CV).



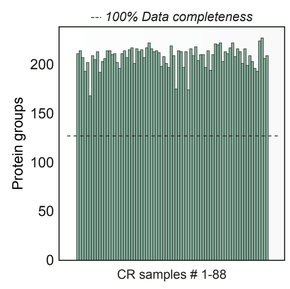
Proteome coverage across SSC samples.



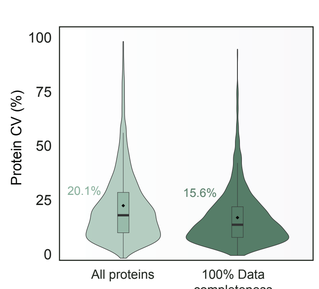
Protein level inter-plate CV.

Common reference (CR)

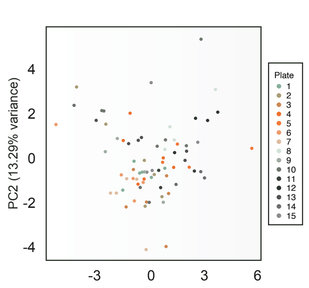
Using pooled patient plasma (CR) samples validates the reliability and precision of the employed workflow.



Proteome coverage across CR samples.



CVs on protein level.



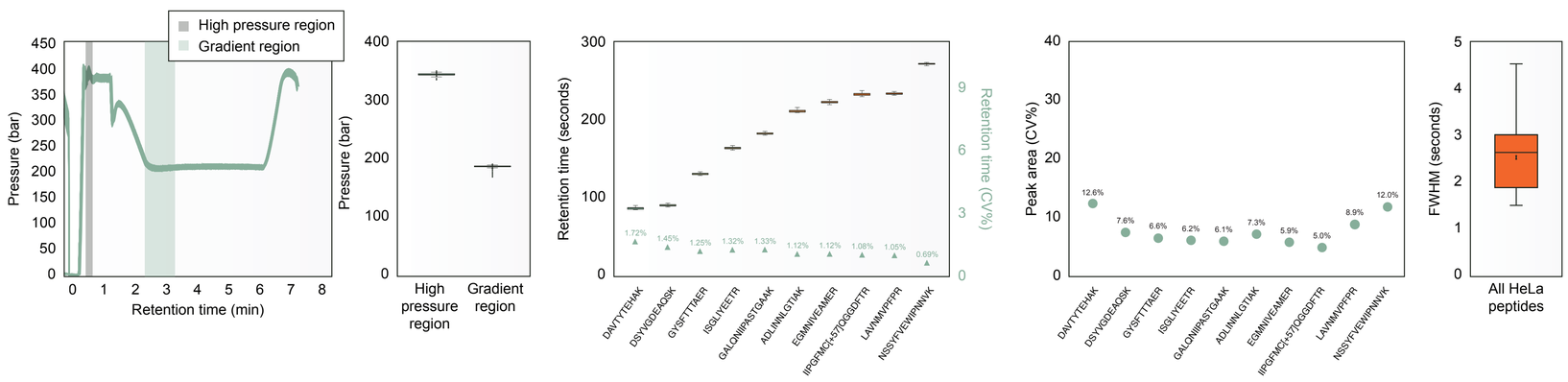
PCA analysis to identify potential batch effect across samples plates.

Unmatched robustness & performance of Evosep One across 1,100+ patient plasma samples

Setting the standard for performance excellence

When analyzing large plasma sample cohorts, the durability and reliability of LC instrumentation is critical. The Evosep One system demonstrated outstanding robustness across all 1,392 samples maintaining stable pressure (<1% CV) throughout.

HeLa SSC samples confirmed reproducible chromatographic performance, with high retention time consistency (<2% CV), stable peak area measurements (<8% CV) and FWHM metrics. This ensured high-quality separation, reproducibility, and accurate quantification, making Evosep One the ideal solution for large-scale proteomics studies requiring operational stability.



Pressure profiles for all samples analyzed with 200 SPD on Evosep One (n = 1,392); assessment of variation in high-pressure region (grey) and gradient region (green) of all analyses, Boxplot of retention time and peak area CV% for elected HeLa SSC peptides (n =10) across all HeLa SSC samples (n = 88), FWHM of all detected HeLa peptides (n = 1.005.000) across all HeLa SSC samples (n = 88).