Deep biofluid proteomics for analysis of large patient cohorts

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Introduction: Mass spectrometry-based Clinical Proteomics

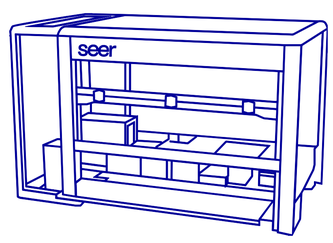
Evotec offers Clinical Proteomics services for biofluids such as plasma, serum, cerebrospinal fluid (CSF) or urine employing the Proteograph XT™ platform, a nanoparticle-based technique. Here, we present results on the long-term stability of the Proteograph XT™ workflow with a focus on multi-centric patient studies.

E.MPD
Evotec Molecular Patient Database

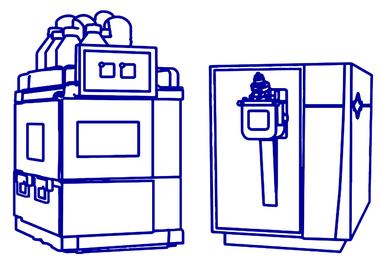
PanOmics
Data generation

PanHunter
Interactive Omics Analysis

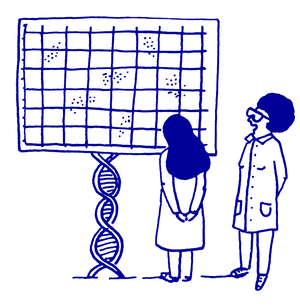
EVOgnostic
Machine Learning based models



Proteograph platform for preparation of body fluids (e.g. plasma, serum, urine, CSF) using nanoparticle-based enrichment



High throughput, high resolution measurements for peptide and protein identification and quantification



Data obtained from Evotec's industrialized clinical proteomics workflow can serve multiple purposes, including the discovery of novel biomarkers, enhancing disease understanding, stratifying patients, and development of machine learning models for improved diagnosis of complex diseases.

High-complexity and dimensionality of data perfectly suited for building of machine learning based models and hypothesis-free unbiased data generation

Quality control data acquired during measurement of multiple large patient cohorts to test for sample quality and long-term stability

Example 1: Quality control for serum or plasma samples

Standardized workflow for inspection of samples and correlation with information from Mass Spectrometry measurement

For multi-centric studies and large cohorts where samples have been collected over a long period of time, the correct correlation of sample properties (e.g. erythrocyte or platelet contamination level, coagulation status) with proteomics data is very important. For the data interpretation it is crucial to consider, that both disease status and conditions of blood withdrawal can have an influence on biofluid properties.

At Evotec, we have extensive experience with interpretation of serum or plasma data from large clinical patient cohorts. This is especially important, when samples from multiple clinical cohorts are combined.

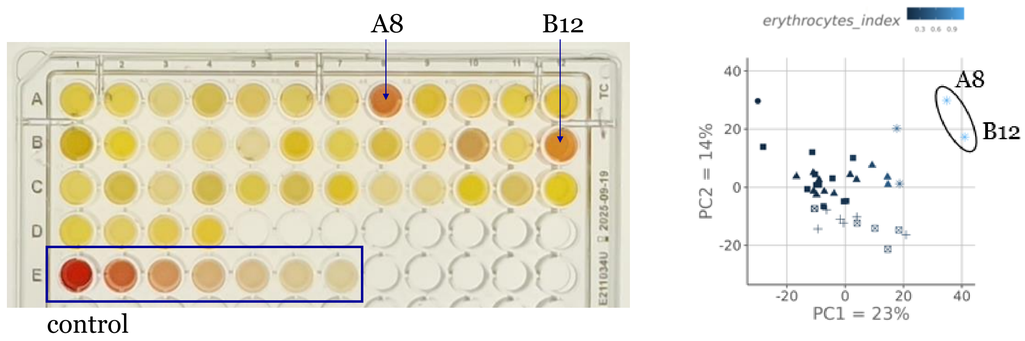


Figure 1: Combining visual QC analysis with MS data. As an example, serum samples from four different disease cohorts are shown (left). For the same samples a principal component analysis (PCA) of the proteomics data acquired by mass spectrometry is shown (right), indicating that indeed samples with high visual contamination level (A8, B12) can be identified as outliers.

Example 2: Long-term stability of Mass Spectrometry platform

Stability of the Proteograph platform is assessed using pooled control samples

Platelet poor plasma samples pooled from 5 male and 5 female healthy donors (BioIVT) were used to monitor performance stability of the Proteograph platform in a large-scale clinical study with over 500 samples and across 10 measurements plates (P1-P10). The Proteograph platform achieves excellent reproducibility highlighted by a constant number of protein group identifications and high quantitative precision with over 900 protein groups being identified at a CV < 20%.

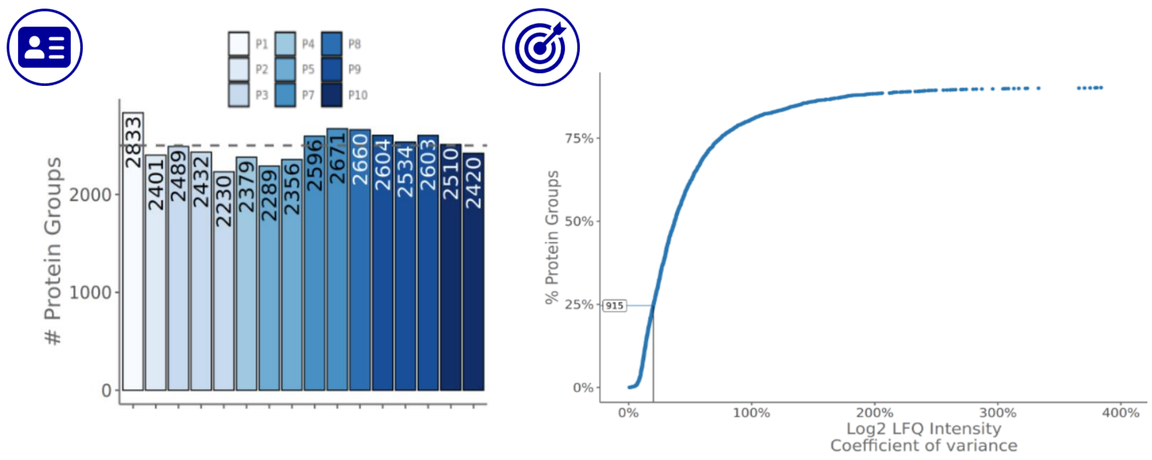


Figure 2: Stability of Proteograph XT platform in large-scale clinical study. Number of protein group identifications and quantitative precision across 10 different plates with 40 clinical samples each that were measured at various points over 3 months.

What are the advantages of Mass Spectrometry-based analysis of biofluids over other technologies like affinity-based proteomics?

Combining nanoparticle-based enrichment of biofluids with Mass Spectrometry enables, comprehensive intensity data generation for thousands of proteins in each patient sample. Howeverit is important to recognize that while affinity-based methods using antibodies or aptamers to bind specific proteins, are hypothesis-driven, Mass Spectrometry-based measurements are entirely hypothesis-free. This allows for the detection of truly novel signals and post-translational modifications, such as phosphorylations.

In addition, it is also possible to generate insight into how protein quantitative trait loci predicted from genome wide association analysis influence protein quantity, without being confounded by potential epitope effects.

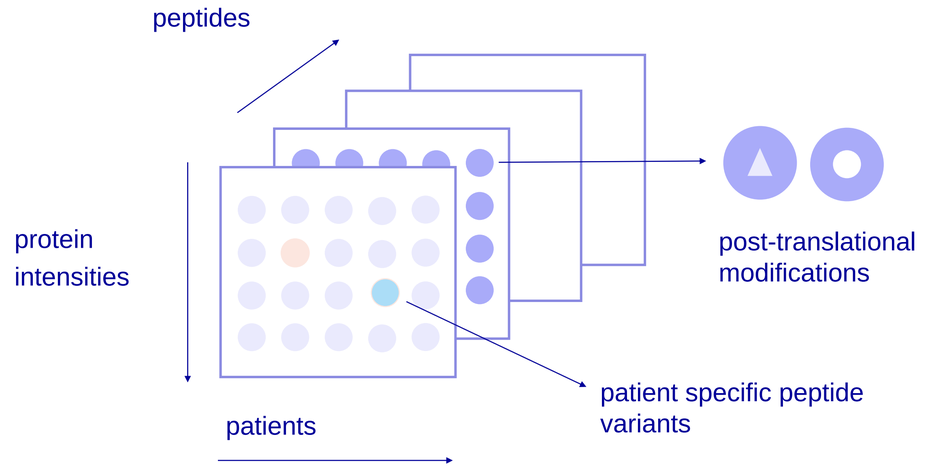


Figure 3: Depth of MS Proteomics data. Up to 100s of peptides per protein and multiple potential post-translational modifications per peptide can be identified.

Conclusion

At Evotec, we have developed a workflow that combines the enrichment of proteins from clinical biofluids using nanoparticles with high-end Mass Spectrometry measurements. This method is robust and stable enough to measure large patient cohorts

Dive deeper into Clinical Proteomics by watching our joint Evotec and Seer Webinar
https://news.evotec.com/joint-evotec-seer-webinar-ondemand
https://www.evotec.com/en/panomics-technology-platforms/proteomics

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