

# Multicenter evaluation of label-free quantification in human plasma on a high dynamic range benchmark set

## Info

PUBLIZIERT AM      JOURNAL  
2025-10-02      Nature Communications

## LINK

<https://doi.org/10.1038/s41467-025-64501-z>

## Autoren

Ute Distler   Han Byul Yoo   Oliver Kardell   Dana Hein  
Malte Sielaff   Marian Scherer   Anna M. Jozefowicz  
Christian Leps   David Gomez-Zepeda  
Christine von Toerne   Juliane Merl-Pham  
Teresa K. Barth   Johanna Tüshaus   Pieter Giesbertz  
Torsten Müller   Georg Kliewer   Karim Aljakouch  
Barbara Helm   Henry Unger   Dario L. Frey  
**Dominic Helm**   Luisa Schwarzmüller   Oliver Popp  
Di Qin   Susanne I. Wudy   Ludwig Roman Sinn  
Julia Mergner   Christina Ludwig   Axel Imhof  
Bernhard Kuster   Stefan F. Lichtenthaler  
Jeroen Krijgsveld   **Ursula Klingmüller**   Philipp Mertins  
Fabian Coscia   Markus Ralser   Michael Mülleider  
Stefanie M. Hauck   Stefan Tenzer

## Abstract

Human plasma is routinely collected during clinical care and constitutes a rich source of biomarkers for diagnostics and patient stratification. Liquid chromatography-mass spectrometry (LC-MS)-based proteomics is a key method for plasma biomarker discovery, but the high dynamic range of plasma proteins poses significant challenges for MS analysis and data processing. To benchmark the quantitative performance of neat plasma analysis, we introduce a multispecies sample set based on a human tryptic plasma digest containing varying low level spike-ins of yeast and E. coli tryptic proteome digests, termed PYE. By analysing the sample set on state-of-the-art LC-MS platforms across twelve different sites in data-dependent (DDA) and data-independent acquisition (DIA) modes, we provide a data resource comprising a total of 1116 individual LC-MS runs. Centralized data analysis shows that DIA methods outperform DDA-based approaches regarding identifications, data completeness, accuracy, and precision. DIA achieves excellent technical reproducibility, as demonstrated by coefficients of variation (CVs) between 3.3% and 9.8% at protein level. Comparative analysis of different setups clearly shows a high overlap in identified proteins and proves that accurate and precise quantitative measurements are feasible across multiple sites, even in a complex matrix such as plasma, using state-of-the-art instrumentation. The collected dataset, including the PYE sample set and strategy presented, serves as a valuable resource for optimizing the accuracy and reproducibility of LC-MS and bioinformatic workflows for clinical plasma proteome analysis.