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APPS LAB

Unbiased and deep proteomic analysis of complex biofluids using the Proteograph® ONE Assay

Maedeh Zamani, Dashyanng Kachru, Shao-Yung (Eric) Chen, Susan Dang, Adam Brewer, Fredric Murolo, Xiaoyan Zhao, Aaron S. Gajadhar

Introduction

Proteomic analysis of complex biofluids, including conditioned media (CM) and cerebrospinal fluid (CSF), provides transformative opportunities for advancing our understanding of human health, disease mechanisms, and biomarker discovery. Both CM and CSF are rich in secreted proteins and signaling molecules that serve critical roles in cell–cell communication, tissue homeostasis, and disease progression. However, these sample types also present substantial analytical challenges.

CM secretomes provide a valuable window into the extracellular environment of cells and represent an important class of samples for studying intercellular signaling, drug target identification, therapeutic development, and the discovery of potential biomarkers. Their analysis, however, is limited by a vast dynamic range of protein concentrations and the masking effects and interference from highly abundant proteins from cell culture supplements such as fetal bovine serum (FBS). Conventional workflows typically require extensive preprocessing steps such as ultrafiltration, fractionation, or protein depletion, and often employ serum starvation to reduce background signals. While these methods improve coverage, they are time- and labor-intensive, introduce variability, and risk perturbing the native biology of the secretome.

CSF is also a highly informative yet analytically challenging biofluid. It circulates within the central nervous system (CNS), offering a direct biochemical link to neurological function and disease. CSF contains a wide spectrum of proteins, including neurodegenerative disease biomarkers, signaling molecules, and other low-abundance factors that are difficult to capture with conventional proteomic techniques. Traditional methods for CSF analysis often demand extensive concentration or fractionation steps, and can still fail to detect proteins of high clinical and biological relevance. These limitations are particularly problematic in translational and preclinical studies, where sample availability is inherently constrained—such as in small-volume CSF collections from model organisms.

The Proteograph ONE Assay platform applies nanoparticle-based unbiased protein capture to overcome these obstacles in both CM and CSF. By enabling scalable, reproducible, and comprehensive proteome coverage without the need for disruptive preprocessing, the Proteograph ONE workflow expands detection to low-abundance, disease-relevant proteins. Here, we demonstrate its application in CM and CSF analyses, demonstrating deeper coverage, superior reproducibility, and enhanced detection of biologically meaningful proteins compared to conventional direct digestion workflows.

About this application note:

Conditioned media (CM) and cerebrospinal fluid (CSF) are highly informative sample types for proteomic analysis, offering insights into cell–cell signaling, intercellular communication, and neurological disease biology. In this application note, we evaluated the application of the Proteograph® ONE workflow, an unbiased nanoparticle-based deep proteomics platform, to systematically characterize both CM and CSF biofluid proteomes. Using a standardized protocol without additional preprocessing or depletion steps, we demonstrate the feasibility of comprehensive proteomic characterization across diverse CM conditions and CSF sample volumes. The results demonstrate how the Proteograph ONE workflow enables researchers to obtain high-resolution, reproducible proteomic data from complex and limited-volume biofluids, facilitating biomarker discovery and translational research.

Study Design

Sample Information

Conditioned Media: CM samples werre collected from multiple cell lines cultured under various conditions. HeLa cells (DMEM, 0%, 2%, and 10% FBS, supplemented with 1% Penicillin- Streptomycin), BT-549 cells (RPMI 1640, 0%, 2%, and 10% FBS, supplemented with 1% Penicillin-

Streptomycin), and human iPSCs (mTeSR1 basal medium with supplement) were analyzed (n=3 replicates per condition).

Cerebrospinal Fluid: CSF samples were obtained from three healthy human donors, and each was assayed across varying input volumes of 10, 25, 50, and 120 µL (n=4 per donor). A pooled rat CSF sample (n=4 replicates) was also analyzed to evaluate species-agnostic profiling.

Sample Preparation

Samples were processed with the Proteograph® Product Suite using the Proteograph ONE Assay shown in Figure 1. 120 µL of the samples were mixed with the nanoparticles suspension, and proteins were quantitatively captured in protein coronas. For low volume samples, the indicated volume of the sample was mixed with the Proteograph ONE Assay Binding Solution to yield a final volume of 120 µL, before mixing with the nanoparticles suspension. Captured proteins were subsequently denatured, reduced, alkylated, and subjected to proteolytic digestion (trypsin and Lys-C). Peptides were purified and yields were determined. All the steps were completed on the SP200 Automation Instrument. Peptides were then dried down overnight and subsequently reconstituted in a solution of 0.1% formic acid and 3% acetonitrile spiked with 5 fmol/µL PepCalMix from SCIEX (Framingham, MA, USA) on the SP200 Automation Instrument before injection onto the LC-MS system.

Data-Independent Acquisition (DIA) Mass Spectrometry

For data-independent acquisition (DIA), samples were analyzed with a Vanquish NEO nanoLC system coupled with an Orbitrap™ Astral™ (Thermo Fisher Scientific) mass spectrometer using a trap-and-elute configuration. First, the peptides were loaded onto an Acclaim™ PepMap™ 100 C18 (0.3 mm ID x 5 mm) trap column (Thermo Fisher Scientific) and then separated on a Gen1 50 cm µPAC™ analytical column (Thermo Fisher Scientific) at a flow rate of 1 µL/min using a gradient of 4-35% mobile phase B (acetonitrile/0.1% formic acid) mixed into mobile phase A (water/0.1% formic acid) over 21 minutes, resulting in a 26-minute run time. The mass spectrometer operated in DIA mode. MS1 scans were performed in the Orbitrap detector at 240,000 resolution, every 0.6 seconds with a 5 ms ion injection time or 500% automatic gain control (AGC; 500,000 ion) target. For the DIA experiment, two hundred fixed DIA windows covering a 380–980 m/z mass range were collected at the Astral detector per cycle with 3 Th precursor isolation windows. MS2 scans were collected from 150-2000 m/z.

Data Analysis

DIA data were processed using Proteograph® Analysis Suite (PAS). The spectra were processed using the DIA-NN search engine (version 1.8.1) in library-free mode searching MS/MS spectra against an in silico generated spectral library from combined entries from Uniprot bovine (UP000009136_9913) and human (UP000005640_9606) databases for CM, (UP000005640_9606) database for human CSF, and (UP000002494_10116) for rat CSF. Library-free search parameters included trypsin protease, 1 missed cleavage, N-terminal methionine excision, fixed modification of cysteine carbamidomethylation, peptide length of 7-30 amino acids, precursor range of 300-1800 m/z, and fragment ion range of 200-1800 m/z. MS1 and MS2 mass accuracy was set to 3 and 8 ppm, respectively. Precursor and Protein Group false-discovery rate (FDR) thresholds were set at 1%. Qualification was performed on summed abundances of all unique peptides considering only precursors passing the FDR thresholds.

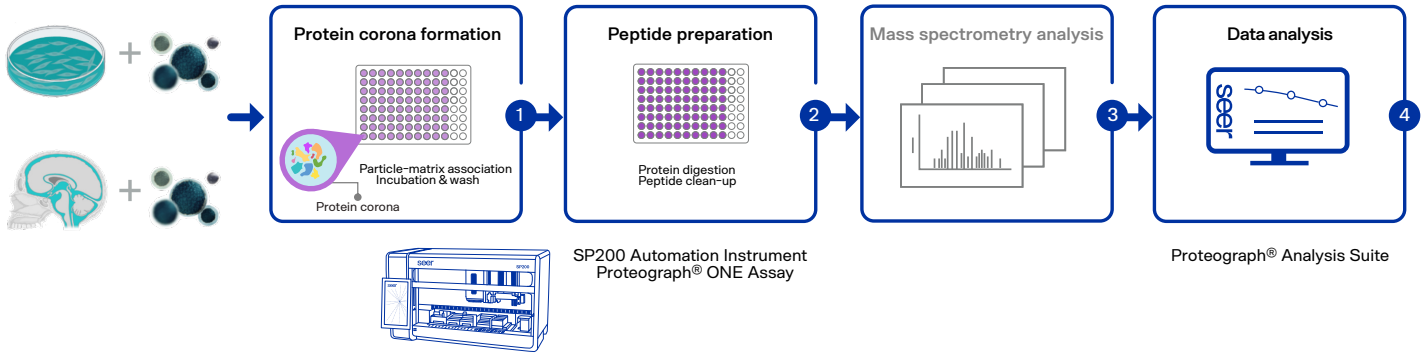


Figure 1. The Proteograph ONE workflow. (1) Samples are added to Seer's NP suspension and a protein corona is formed. (2) Isolated proteins are processed, and the resulting peptide mixture is desalted and quantified. (3) Peptides are injected onto an LC-MS system for unbiased proteome analysis. (4) LC-MS raw data are transferred to Proteograph Analysis Suite (PAS) for peptide and protein identification.

The human secretome from Human Protein Atlas (https://www.proteinatlas.org/humanproteome/tissue/secretome#classification, accessed July 2025) was used as the reference to identify the annotated secretome in conditioned media samples, using a compiled list of all human and human/bovine shared proteins detected across all samples.

To identify neurology-related proteins, more than 20,000 human proteins in the UniProt database were analyzed against the OpenTargets database for disease associations based on protein-specific evidence. Diseases were mapped to ICD-10 chapters for structured classification, and evidence thresholds were applied. Over 1,200 proteins with neurology-related evidence scores higher than 0.1 were identified. The list was used as the reference for identifying neurology-related proteins detected in human CSF.

Results

We evaluated the performance of the Proteograph ONE Assay in two analytically-challenging biofluids: conditioned media (CM) and cerebrospinal fluid (CSF). The following sections describe gains in proteome coverage, species-specific resolution, and secretome characterization in CM, as well as the ability to achieve comprehensive CSF profiling across varying input volumes. In addition, we highlight the reproducibility of the workflow and its capacity to broaden the detection of disease-relevant biomarkers.

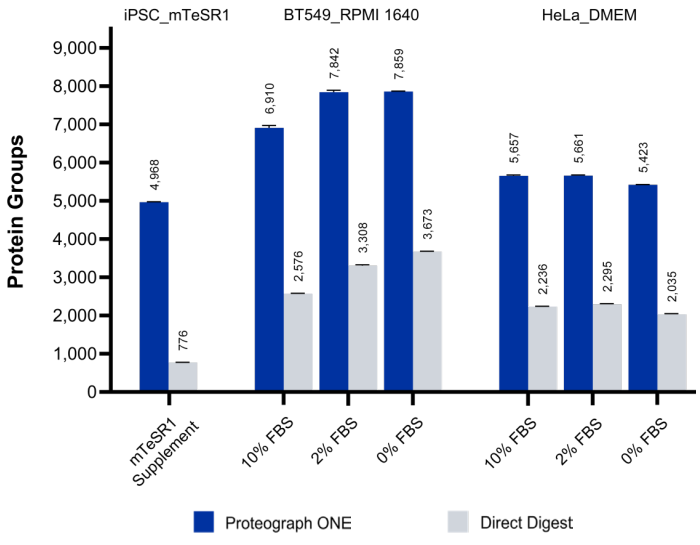


Figure 2. Protein identifications in conditioned media with cell culture supplement and varying levels of FBS, using the standard Proteograph ONE workflow with standard assay input volume compared to the direct digestion workflow. Mean protein identification numbers are shown, with error bars indicating standard deviation (n = 3).

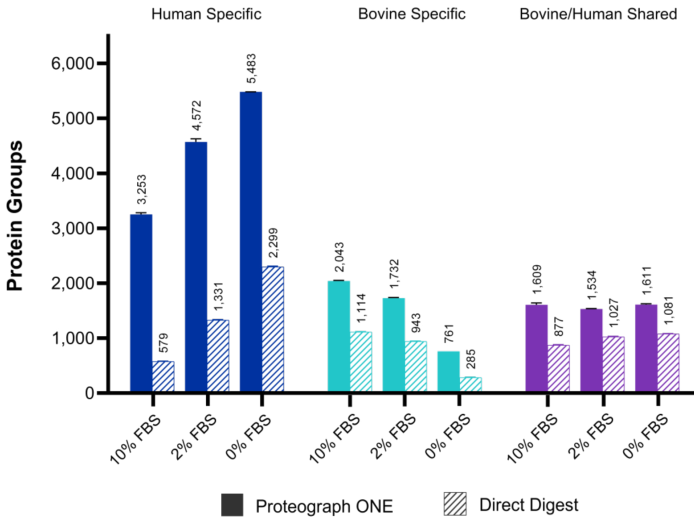


Figure 3. Human- and bovine-derived protein identifications obtained with the Proteograph ONE Assay in conditioned media from the representative cancer cell line BT549, cultured in RPMI 1640 with varying levels of FBS, compared to direct digestion.

Conditioned Media

Proteograph ONE Assay Achieves Superior Depth of Proteome Coverage

To assess the efficacy of the Proteograph ONE workflow relative to direct digestion, we conducted a comparative analysis of proteome identifications. Across all tested secretome contexts, Proteograph ONE Assay delivered 2.1–6.4× greater proteome coverage than direct digestion (Figure 2). This advantage persisted regardless of FBS level or media formulation, eliminating the need for conventional manipulations like serum starvation or protein depletion. Even in 48-hour serum-deprived conditions (0% FBS), Proteograph ONE Assay expanded the proteome coverage compared to direct digestion, indicating that the platform's depth is not contingent on serum removal. In practical terms, the nanoparticle-based capture increased access to secreted proteins that are typically masked by highly abundant serum proteins in conventional workflows. Furthermore, the approach was broadly compatible with multiple media formulations including DMEM, RPMI 1640, and mTeSR1 and their supplements, simplifying experimental setup and enabling consistent discovery across cell culture models.

A key challenge in serum-containing CM is the dominance of bovine proteins originating from FBS, which can obscure secreted proteins of interest (i.e., human proteins in this case) and bias downstream conclusions. Proteograph ONE Assay markedly increased the proportion of detected human proteins while reducing the contribution from unwanted

bovine proteins (Figure 3). This improvement was noted for all FBS levels (10%, 2%, or CM samples subjected to a 48-hour FBS starvation regimen), demonstrating that nanoparticle-based capture of the secretome is resilient to FBS background and media composition.

Notably, in high-FBS samples (10% FBS), where direct digestion was dominated by bovine proteins, Proteograph ONE Assay achieved more than a 5× improvement in human secretome coverage. This enrichment of secreted proteins translates directly into higher-confidence biological interpretation and reduces the need for laborious depletion schemes or starvation protocols that can introduce artifacts to the underlying biology.

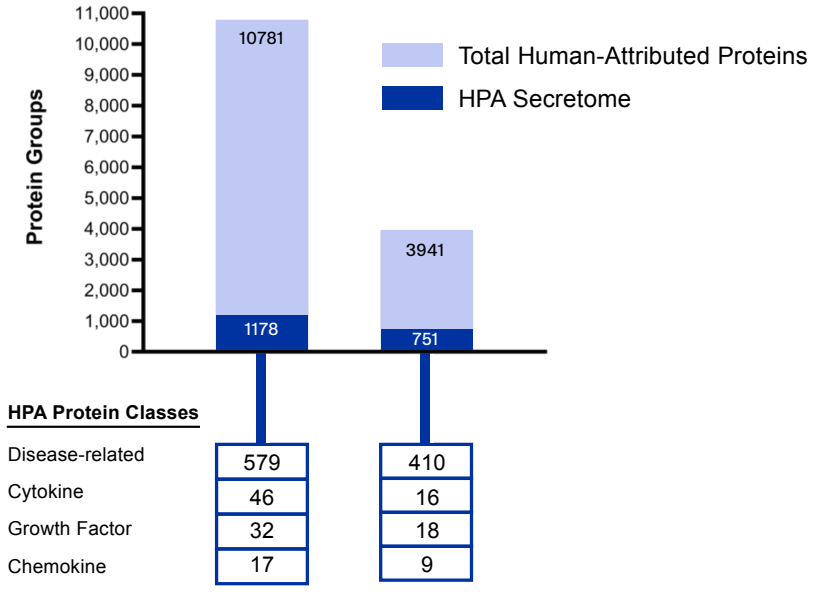
Expanded Access to the Human Secretome

Beyond species-specific improvements, Proteograph ONE Assay extended coverage deep into the human secretome. Approximately half of the HPA-annotated secretome (1,178 of 2,472 proteins) was quantified, representing a ~57% increase compared to direct digestion (Figure 4a). This extended coverage enabled identification of hundreds of disease-relevant, low-abundant secreted proteins, including cytokines and growth factors that are often underrepresented with conventional methods (Figure 4b). For example, cytokines such as IL-6 and CXCL8 and growth factors like VEGFA were detected by both direct digestion and Proteograph ONE Assay, serving as important baseline markers of secretome detection. Critically, Proteograph ONE Assay identified additional low-abundance secreted proteins exclusive to its workflow, including CXCL10 and PDGF-B, which serve roles in immune modulation and tissue regeneration. The ability to capture these biologically meaningful proteins provides a more comprehensive view of the cellular secretome, supporting downstream applications in biomarker discovery, therapeutic target identification, and mechanistic studies of cell–cell communication.

Reproducibility and Quantitative Performance

Proteograph ONE Assay delivered precise and reliable quantification across all CM conditions. Protein intensity coefficients of variation (CVs) were consistently below 12% (Figure 5a), and inter-replicate protein identification overlap was high, with Jaccard indices ranging from 0.95 to 0.97 (Figure 5b). This level of reproducibility demonstrates that the workflow produces minimal technical variance and ensures high-fidelity measurement of biological signals. Such reproducibility is essential for robust differential analyses, pathway interpretation, and prioritization of secreted candidates for follow-up studies.

a)



b)

IL6	CXCL6	PDGFB	NGF	BMP2
IL11	CXCL8	PDGFC	NTF4	BMP4
IL17C	CXCL10	PDGFD	MANF	BMP6
LIF	CXCL12	PGF	MDK	BMP7
MIF	CXCL16	KITLG	PTN	BMP10
CCL2	LEFTY1	GDF5	SPP1	ORM2
CCL5	LEFTY2	GDF6	NAMPT	SAA1
CCL7	VEGFA	GDF15	GRN	NRG1
CCL8	VEGFB	GDF11	HDGF	CSF1
CCL20	VEGFC	BDNF	FN1	CSF3
CCL26	FGF2	TIMP1	SERPINA3	TNFSF12
CXCL1	FGF4	THBS4	TGFB2	TNFSF13
CXCL2	FGF8	CRP	TGFB3	TNFSF15
CXCL3	HGF	HP	SAA4	GREM1
CXCL5	PDGFA	ORM1	CER1	GREM2

Unique to Proteograph ONE Assay

Figure 4. Human Protein Atlas (HPA) secretome analysis showing total secretome identifications and high-value protein classes measured by the Proteograph ONE Assay and by direct digestion. (a) Human-attributed proteins were compiled across all CM conditions, excluding bovine-exclusive proteins, and referenced against the HPA secretome database. (b) Representative cytokines, chemokines, and growth factors detected in CM, with teal indicating proteins uniquely identified by the Proteograph ONE Assay.

Multivariate analyses further highlighted the assay's reliability: principal component analysis (PCA) identified that biological replicates clustered tightly together, while different cell lines and serum concentrations were clearly separated (Figure 6). This demonstrates that Proteograph ONE Assay not only maintains reproducibility but also captures the biologically driven protein abundances inherent to each condition. The platform resolved distinct protein abundance signatures for different cell lines and FBS levels. These high-resolution profiles identified nuanced expression changes across cell culture models, enabling confident discrimination of cell-type- and condition-specific secretome features. The ability to resolve such subtle yet biologically meaningful differences underscores the value of Proteograph ONE Assay in uncovering the complexity of secreted proteomes.

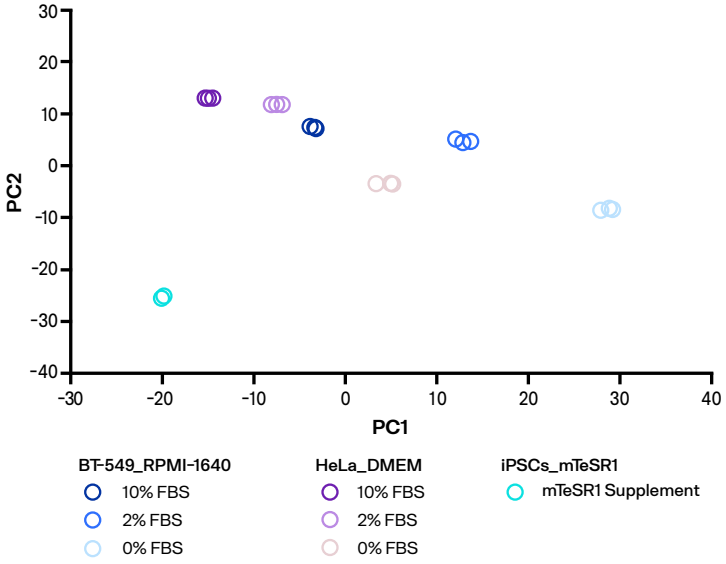


Figure 6. Principal component analysis (PCA) of differential proteomic profiles across all conditioned media samples (n = 3 replicates per condition).

Cerebrospinal Fluid

Proteograph ONE Achieves Deeper Proteome Coverage

Proteograph ONE Assay substantially improved proteome coverage in cerebrospinal fluid samples compared to direct digestion. In human CSF, the workflow delivered 30–80% greater depth at standard input volumes (120 µL) (Figure 7a). Even when input was reduced to just 50 µL, Proteograph ONE Assay maintained a strong advantage, achieving ~20–55% more protein identifications than direct digestion. Coverage improvements were more variable for input volumes < 50 µL and dependent on individual donor sample. However, the inherent flexibility of Proteograph ONE Assay to accommodate a range of CSF sample volumes is particularly important because CSF is a precious and often limited sample type in clinical research. For preclinical studies, where CSF yields from model organisms such as rats are inherently low, the workflow demonstrated similar robustness. With standard 120 µL rat CSF inputs, Proteograph ONE Assay expanded proteome coverage by ~60% (Figure 7b). Remarkably, at only 10 µL of input, the assay identified hundreds of proteins that conventional workflows could not detect. Together, these findings underscore the assay's flexibility for both human and

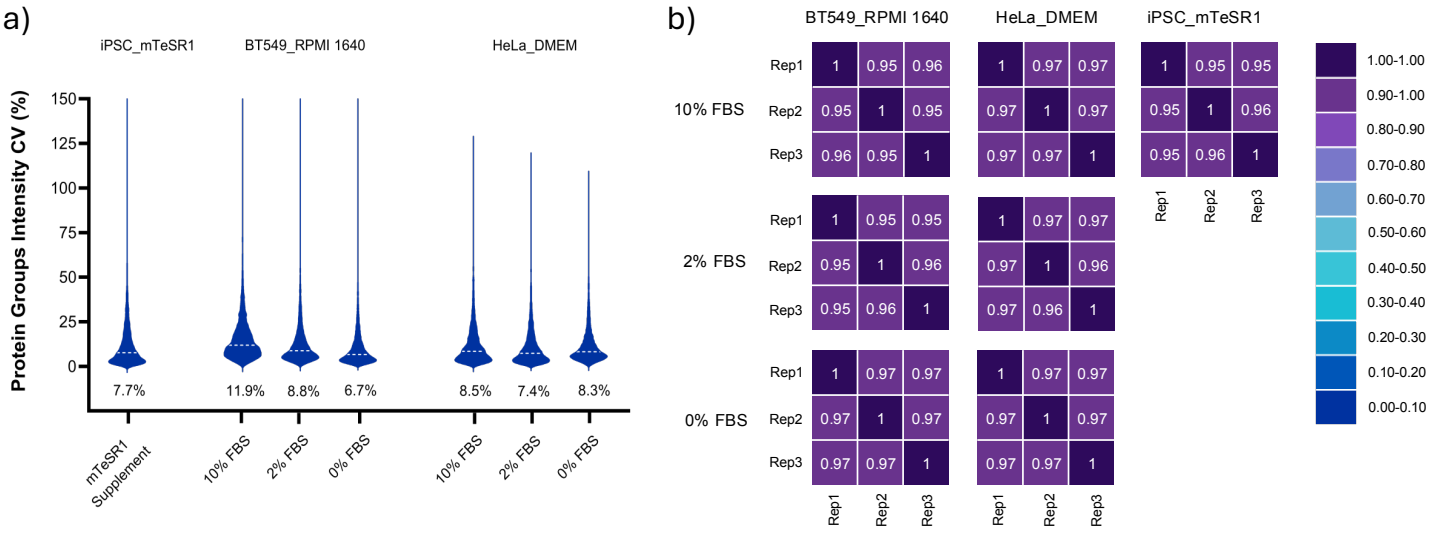


Figure 5. Assessment of the reproducibility of the Proteograph ONE Assay: (a) Inter-replicate intensity coefficient of variation across all consistently measured protein groups. (b) Inter-replicate Jaccard index for protein identifications.

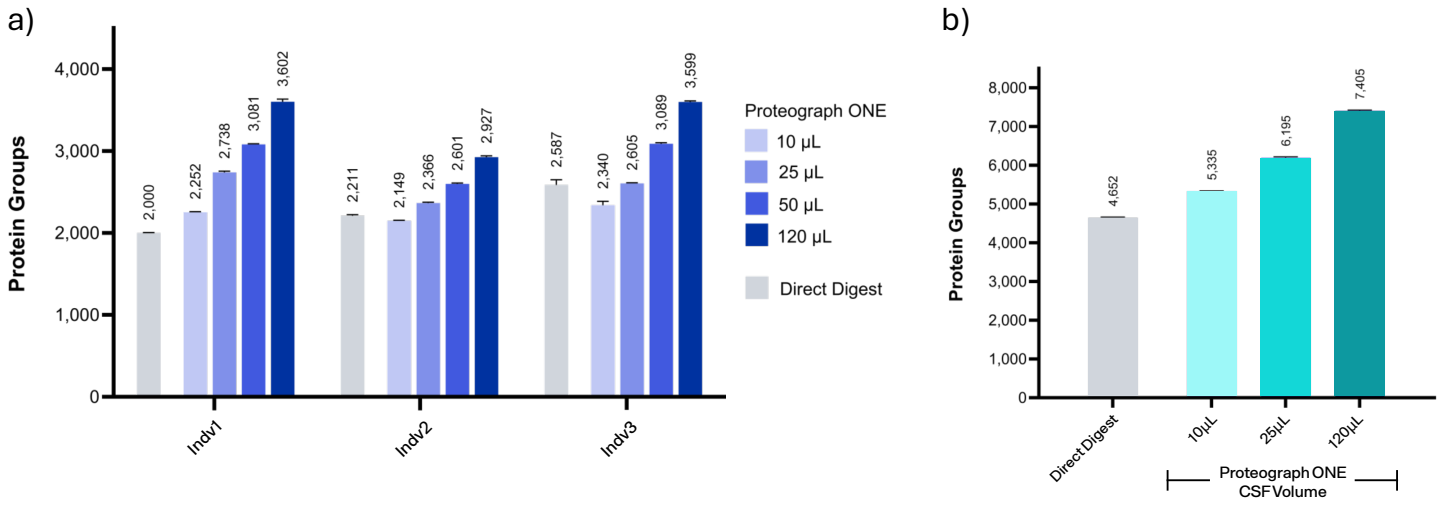


Figure 7. Protein identifications in (a) human CSF and (b) rat CSF across a range of sample input volumes, using the standard Proteograph ONE workflow compared to the direct digestion workflow. Mean protein identification numbers are shown, with error bars indicating standard deviation (n = 4). Indv: individual donor sample.

model organism studies, and its species-agnostic capability to deliver high-resolution proteomic profiles from limited inputs.

Reproducibility and Biomarker Detection

Proteograph ONE Assay produced highly reproducible results across both human and rat CSF, with protein intensity CVs remaining below 16% in human samples and below 11% in rat samples, notably at reduced input volumes (Figure 8a, 8b). This consistency enables confident biological interpretation across donors, dilutions, and species. Beyond consistent measurement, the workflow also broadened detection of neurology-related biomarkers by ~48% relative to direct digestion (Figure 9a). Data from CSF analyses identified dozens of established and emerging neurodegenerative biomarkers. Proteins detected in both direct digestion and Proteograph ONE Assay included APP, MAPT (tau), and SNCA (alpha-synuclein), reflecting core markers of Alzheimer's and Parkinson's disease (Figure 9b). Critically, the Proteograph ONE workflow uncovered additional biomarkers that were exclusive to this method, such as VPS35 and GRIA1, that are reported to be linked to neurological disease pathways, and their detection underscores the platform's ability to illuminate biologically meaningful signals that remain inaccessible to conventional approaches.

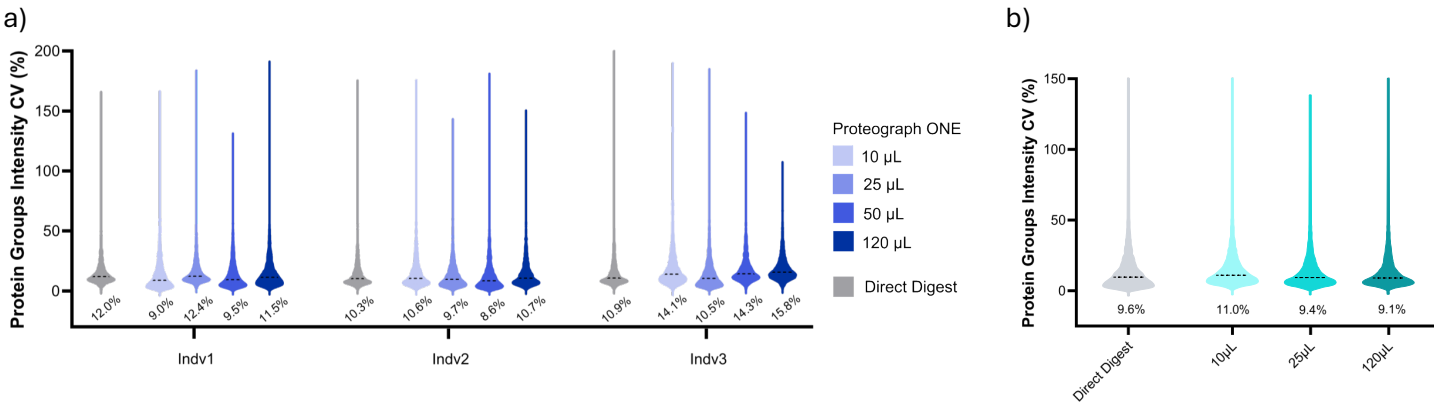
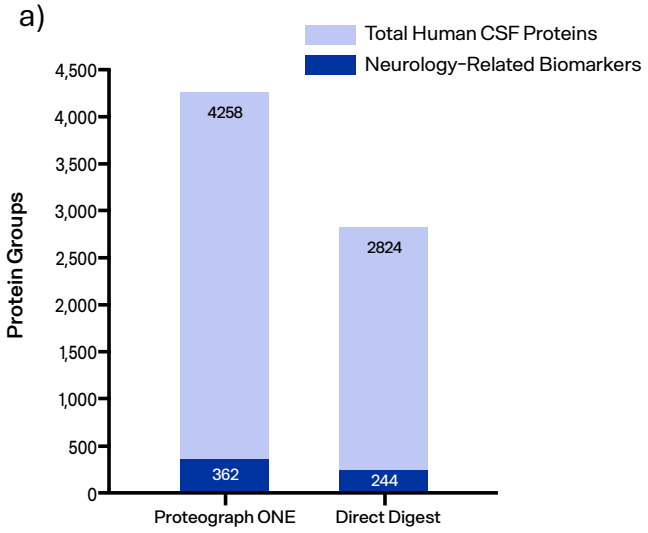


Figure 8. Coefficient of variation of protein group intensities used to assess the quantitative performance of the Proteograph ONE Assay in (a) human CSF and (b) rat CSF across a range of sample input volumes.

a)



b)

Gene Name	Protein Name	Disease Relevance
VPS35	Vacuolar protein sorting 35	PD, AD, ALS[1]
APP	Amyloid-beta precursor protein	AD, PD[2]
MAPT	Microtubule-associated protein tau	AD, PD, ALS[3]
GRIA1	AMPA-type glutamate receptor subunit	AD, ALS, HD[4]
SNCA	Alpha-synuclein	AD, PD[5]
FTL	Ferritin light chain	AD[6]
STUB1/CHIP	E3 ligase co-chaperone	AD[7]
MAPK14	p38α mitogen-activated protein kinase	AD[8]
PARK7	Parkinson disease protein 7	AD, PD[9]
FBXO7	F-box only protein 7	PD[10]
UCHL1	Ubiquitin Carboxyl-Terminal Hydrolase L1	AD, PD[11]
GSK3A	Glycogen synthase kinase-3α	AD[12]
SACS	Sacsin	ARSACS[13]
GFAP	Glial fibrillary acidic protein	AD, PD, ALS[14]

Unique to Proteograph ONE Assay

1) Wu et al.; 2024
2) Ehrenberg et al.; 2018
3) Tracy et al.; 2022
4) Mohallem et al.; 2024
5) Simitsi et al.; 2020
6) Ayton et al.; 2018
7) Sordu et al.; 2025
8) Detka et al.; 2024
9) Karayel et al.; 2022
10) Burchell et al.; 2013
11) Ham et al.; 2021
12) Hampel et al.; 2021
13) Murtinheira et al.; 2022
14) Hansson et al.; 2021

Figure 9. Neurodegenerative disease–related proteins measured by the Proteograph ONE Assay and by direct digestion. (a) Proteins detected in human CSF at standard assay volume were compiled across three donors and referenced against a curated list of >1,200 neurodegenerative disease–related proteins, as described in the Methods section. (b) Representative neurodegenerative disease–related proteins detected in human CSF, with teal indicating proteins uniquely identified by the Proteograph ONE Assay.
Disease Relevance: AD = Alzheimer's disease, PD = Parkinson's disease, ALS = Amyotropic lateral sclerosis, HD = Huntington's disease, ARSACS = Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay

Summary

The Proteograph ONE workflow enables deep, reproducible, and unbiased proteomic profiling of historically challenging biofluids. In CM, the platform consistently increased depth of coverage (2.1–6.4×), shifted detections toward human proteins even in high-FBS contexts (>5× human coverage improvement), and delivered low CVs with strong replicate agreement–without serum starvation or depletion. In CSF, Proteograph ONE Assay provided 30–80% deeper coverage in human samples and ~60% in rat, while maintaining excellent reproducibility and supporting low-input analysis.

The assay's species-agnostic nature expands biomarker detection across both clinical and model organisms, revealing neurology-related proteins—including APP, MAPT, SNCA, VPS35, and GRIA1—several of which remain inaccessible by conventional workflows.

By capturing low-abundance and disease-relevant proteins with high reproducibility, the workflow opens new opportunities for biomarker discovery, therapeutic target identification, and mechanistic insight into human biology and disease. Its flexibility across sample types, input volumes, and species positions Proteograph ONE Assay as a transformative solution for accelerating discoveries in both research and clinical contexts.

Importantly, the Proteograph ONE workflow minimizes the need for traditional preprocessing steps such as serum starvation, depletion, ultracentrifugation, or fractionation. By avoiding these manipulations, it reduces variability and preserves native biology, ensuring that key secreted factors and neurological biomarkers are not lost or distorted in the process. This simplified yet powerful workflow allows researchers to achieve comprehensive proteome coverage in a scalable manner, making it well-suited for large-scale studies and translational pipelines.

Find out more at
seer.bio/products/proteograph-product-suite

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