Nanoparticle Enrichment of Volumetrically Accurate Microsampled Blood for Enhanced Proteomic Analysis of Dried Blood

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INTRODUCTION

Maximizing the interpretive power and utility of proteomic data for Clinical and Precision Health research relies on several key workflow needs:

1. Discovery based workflows that yield **comprehensive proteomes** with **high analytical precision** (low assay CV's).
2. Efficient and consistent workflows capable of handling large sample numbers. This ideally means preparative workflows that are **plate-based**, can be completed within **1-day**, **amenable to automation**, **robust** (inter-day CV's), and **cost effective**.
3. Concordance between plasma and dried blood results, where applicable.

These needs apply to Plasma as well as to Dried Blood, in which an inherently more complex matrix often constrains proteome coverage and limits coverage, analytial precision, and reproducibility.

We surveyed of 4 in-house methods using plasma from patients enrolled in an in-house Alzheimer's research study. We developed and refined a plate-based, 1-day method for both plasma and dried blood, based on the Ceres Peak platform. Finally, we preliminarily evaluated our method for utility for routine, cost effective discovery-based proteomic analysis of clinical samples.

METHODS

Samples: 5mL of venous blood drawn from participants enrolled in the Alzheimer's Disease and Related Dementias program. We simulated microsampling using 96 individual 10uL VAMS devices (Trajan), centrifuged the remaining blood, & aliquoted the plasma.

Primary Metrics: Protein coverage & overlap, intra- &inter-assay variability (CV), Dynamic range, and Protein Completeness.

Phase 1- Survey comparing 4 plasma proteomics methods: We used plasma aliquots from the same patient sample to survey 4 workflows (n=5 aliquots/workflow).

Phase 2- Ceres Performance in plasma and VDBS: We assessed the reproducibility of two Ceres Peak workflows to determine which is more suitable for VDBS.

Phase 3- Digestion time, temperature, & buffer optimization: We used dried blood extract to assess the impact of shorter digestion times on our key performance metrics. We assessed temperature and digestion buffers as key factors towards a 1-day protocol.

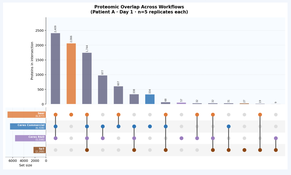
Phase 4- Validated plate-based workflow: We translated the 1-day conditions to a plate-based assay for both plasma and VDBS, focusing on both inter- and intra-day reproducibility (n=5 aliquots/workflow/day, repeated on 3 separate days)

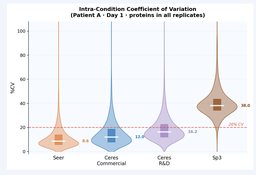
Phase 5- Biological Relevance: Given that the samples used in this study were derived from participants enrolled in an Alzheimer's Disease research study, we interrogated our data to determine the extent to which known markers of neurodegeneration and Alzheimer's disease could be quantified.

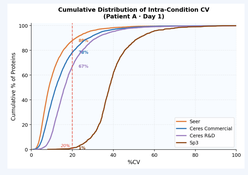
All workflows involved similar extraction (VDBS), reduction, alkylation, and digestion (Trypsin-LysC) steps. Peptides resulting from all replicates for any given phase were acquired by DIA LC-MS/MS in succession using a Vanquish Neo (25cm C18, 20min gradient) coupled to an Orbitrap Astral. Data were searched using a library-free DIA-NN 5.0. (match-between-runs, trypsin/P digestion, 1 missed cleavage allowed, Protein FDR ≤1%; minimum 2 precursors per protein)

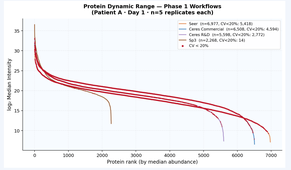
PHASE 1 — SURVEY COMPARING 4 PLASMA PROTEOMICS METHODS

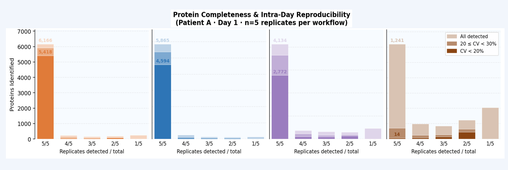
Patient A · n=5 individual aliquots prepared for each of Seer, Ceres Comm., Ceres R&D., and Sp3. All Phase 1 data acquired in random order as a single LC-MS/MS batch

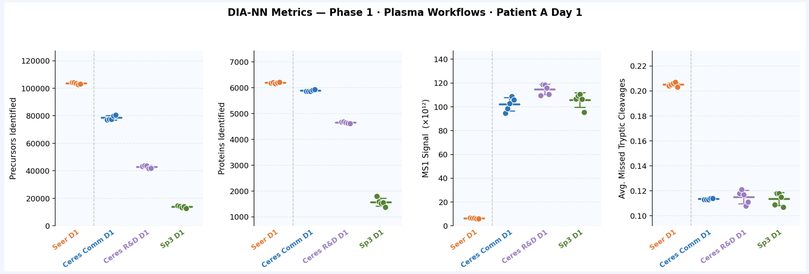










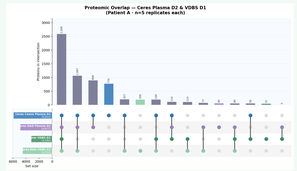


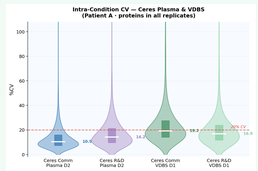
Phase 1 Key Findings

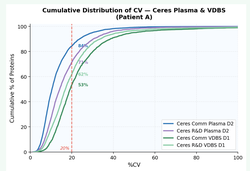
- The Seer Proteograph yields the most proteins (6977), with the lowest average CV (8.6%), and the highest proportion of proteins with CV<20% (88%)
- The Ceres Commercial workflow represents a compelling option for routine cost-effective screening, while the Sp3 workflow performed poorly.
- Precisely measured proteins (CV<20%) are distributed across the dynamic range for both Seer and Ceres-based workflows

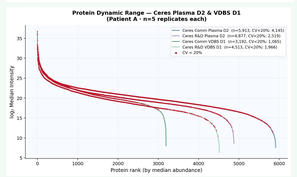
PHASE 2 — CERES PERFORMANCE IN PATIENT PLASMA AND VDBS

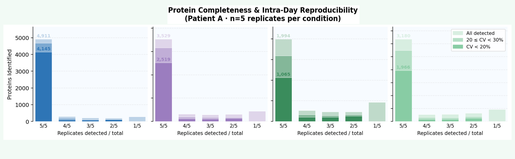
Patient A · Ceres Comm. Plasma vs. Ceres R&D VDBS · n=5 individual plasma and VDBS aliquots prepared in parallel for Ceres Comm., & Ceres R&D. All Phase 2 data acquired in random order as a single LC-MS/MS batch

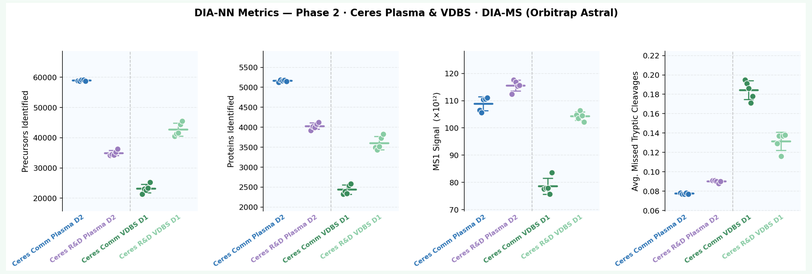










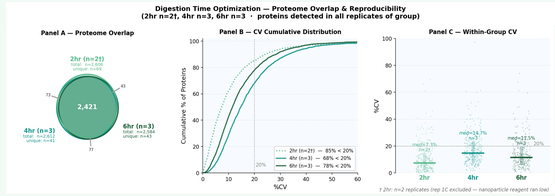


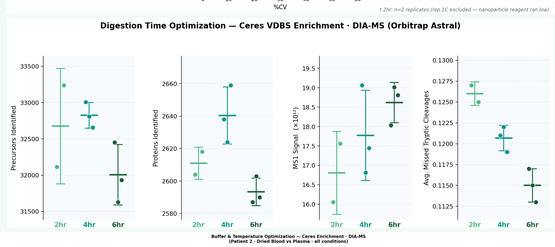
Phase 2 Key Findings

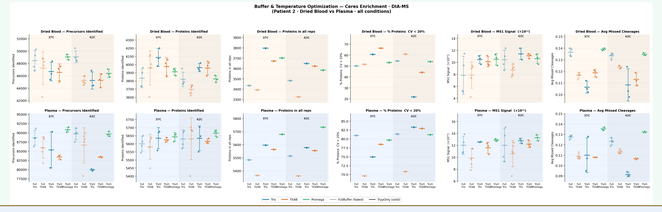
- The Ceres Peak Commercial workflow outperforms the Ceres R&D *for Plasma*, but the Ceres R&D workflow is superior *for VDBS*
- Phase 2 Plasma results strongly agree with the Phase 1 screening assay, suggesting that the Ceres workflow is robust
- VDBS workflows share over 60% of identified proteins with plasma proteome; more than half of VDBS proteins achieve CV<20%

PHASE 3 — DIGESTION TIME, TEMPERATURE, & BUFFER OPTIMIZATION

Patient A VDBS extract · Digestion time evaluation (2, 4, & 6hr) · Patient B Plasma & VDBS extract · Digestion buffer (TEAB, Tris+CaCl₂, Promega) and Temperature (37°C / 42°C) evaluation · All Phase 3 data acquired in random order as a single LC-MS/MS batch





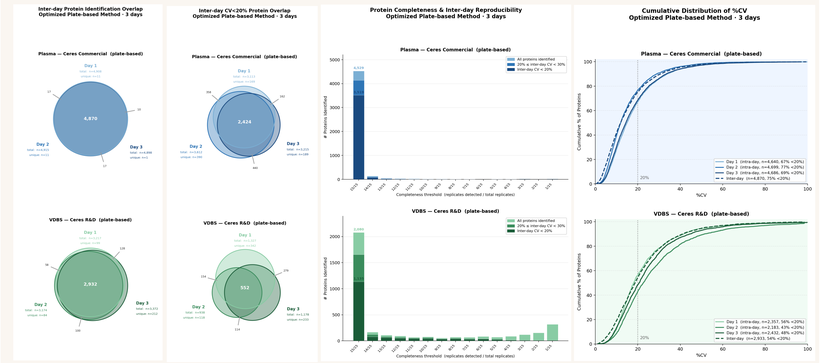


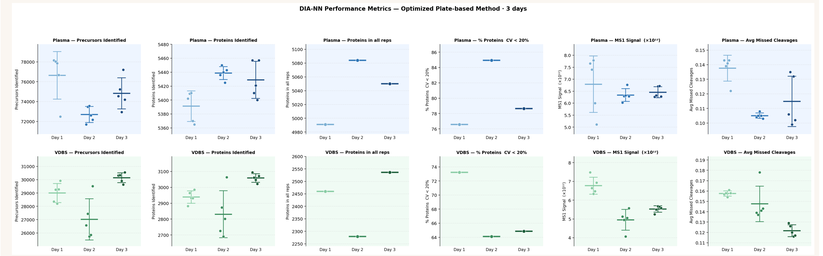
Phases 3 Key Findings

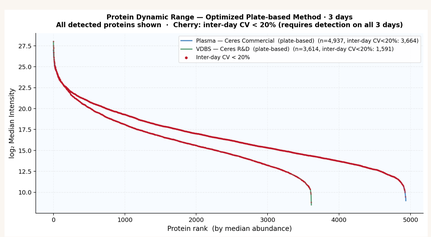
- Protein depth and CV's are comparable between 2, 4, & 6hr protocols with ~50% time savings.
- finalized 2hr digestion protocol may require additional monitoring due to the constraint of only 2 replicates
- TEAB buffer and Tris+$CaCl_2$ at 37°C both seem sufficient for the digestion buffer –though these should be used to resuspend the Trypsin/LysC as opposed to fully used including for resuspending the washed beads
- Final digestion conditions for evaluating a single-day processable for 96 samples: **2 hr · TEAB or Tris+$CaCl_2$ · 37°C** ·

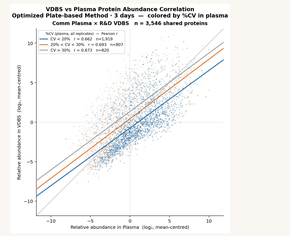
PHASE 4 — VALIDATED PLATE-BASED WORKFLOW: 3-DAY REPRODUCIBILITY

Patient B, Optimized plate-based protocol · n=5 individual plasma and VDBS aliquots prepared in parallel/day × 3 days = 15 independent aliquots in total · All Phase 2 data acquired in random order as a single LC-MS/MS batch









Phase 4 Key Findings

- Plasma (Ceres Commercial) and VDBS (Ceres R&D) perform well as a plate-based, single-day workflow, with good protein coverage and large fractions of proteins with CV<20%
- Results are highly stable across 3 independently prepared acquisition days (>80% of proteins detected in all 3 days; inter-day CV <20% for >60% of detected proteins in both matrices)
- Plasma and VDBS proteomes correlate well (Pearson r = 0.86, for low-CV proteins), supporting cross-matrix quant comparison

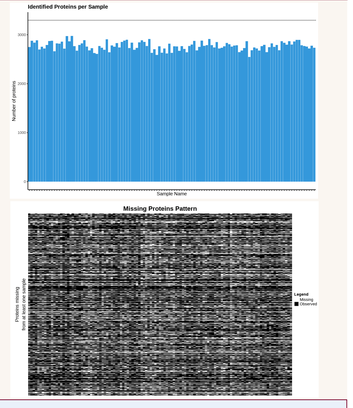
PHASE 5 — BIOLOGICAL RELEVANCE & CLINICAL STUDY UTILITY

Detection of Neurodegenerative Disease Biomarkers

Ceres enrichment of VDBS and Plasma · Label-Free DIA-MS analysis (Orbitrap Astral, 20 min gradient)

CV < 20% · CV 20-30% · CV > 30% / CV n/a · Not detected · ▪ Detected in both patients · % shown = fractional detection

Gene	Description / Disease Association	Plasma	Volumetric Dried Blood Sample (10µL, Neoteryx)	Alzheimer's Disease Relevance
APP	Amyloid precursor protein	**detected in 100%** (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Strong
APOE	Apolipoprotein E (major AD risk gene)	detected in 100% (n=5/5) ▪ both patients	**detected in 100%** (n=5/5) ▪ both patients	Strong
PSEN1	Presenilin-1 (familial AD)	**detected in 40%** (2 patient only)	detected in 0% (not detected)	Strong
ABCA7	ABC transporter A7 (AD GWAS)	**detected in 100%** (n=5/5) ▪ both patients	detected in 33% ▪ both patients	Moderate
CLU	Clusterin / ApoJ (AD GWAS)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Moderate
CFH	Complement factor H (AD GWAS)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Moderate
MAPT	Microtubule-assoc. protein Tau	not detected	not detected	Strong
NEFL	Neurofilament light chain (NfL)	not detected	not detected	Indicative
YWHAZ	14-3-3 ζ (neuronal signaling)	**detected in 100%** (n=5/5) ▪ both patients	**detected in 100%** (n=5/5) ▪ both patients	Indicative
SNCA	α-Synuclein (PD / LBD marker)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Indicative
PARK7	DJ-1 / Parkinsonism deglycase	**detected in 100%** (n=5/5) ▪ both patients	**detected in 100%** (n=5/5) ▪ both patients	Indicative
GFAP	Glial fibrillary acidic protein	not detected	not detected	Moderate
TREM2	TREM2 (microglial AD risk gene)	detected in 40% (2 patient only)	detected in 3% (1 patient only)	Strong
CHI3L1	YKL-40 (astrocyte / neuroinflammation)	detected in 40% ▪ both patients	detected in 97% ▪ both patients	Moderate
GRN	Progranulin (FTD / AD modifier)	detected in 100% (n=5/5) ▪ both patients	detected in 47% ▪ both patients	Indicative
PRNP	Prion protein (prion diseases)	**detected in 100%** (n=5/5) ▪ both patients	detected in 70% ▪ both patients	Indicative
SOD1	Superoxide dismutase 1 (ALS)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Indicative
CST3	Cystatin C (neuroinflammation)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Indicative
PTK2B	Protein-tyrosine kinase 2β (AD GWAS)	**detected in 100%** (n=5/5) ▪ both patients	detected in 80% ▪ both patients	Moderate
IGFBP2	IGF-binding protein 2 (AD / MCI)	detected in 100% (n=5/5) ▪ both patients	detected in 100% (n=5/5) ▪ both patients	Indicative



Phase 5 Key Findings

- 25 of 31 curated AD-relevant proteins detected; 6 detected exclusively in VDBS (complement/coagulation factors)
- Brain-enriched proteins (HPA atlas) detected in both matrices; α-synuclein (SNCA) highly ranked in VDBS — consistent with dried whole-blood origin and potential AD relevance
- In a Heart Disease cohort of 115 individual participants, the VDBS assay consistently yields over 2500 proteins, with a high degree of completeness

CONCLUSIONS

In keeping with our findings and the literature, the Seer Nanoparticle enrichment platform is the gold standard for protein coverage and analytical precision. The Ceres Peak platform however represents a cost-effective complementary or alternative for plasma or VDBS proteomic analysis, and can be implemented as an optimized 2 hr plate-based single-day workflow (TEAB at 37°C). Inter-day validation confirms its robust reproducibility and strong cross-matrix correlation (Pearson r >0.86). Biological validation in ADRD samples confirms 25/31 AD-relevant biomarkers and pathway-level signal from VDBS — establishing a scalable, high-throughput platform for clinical proteomics.

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