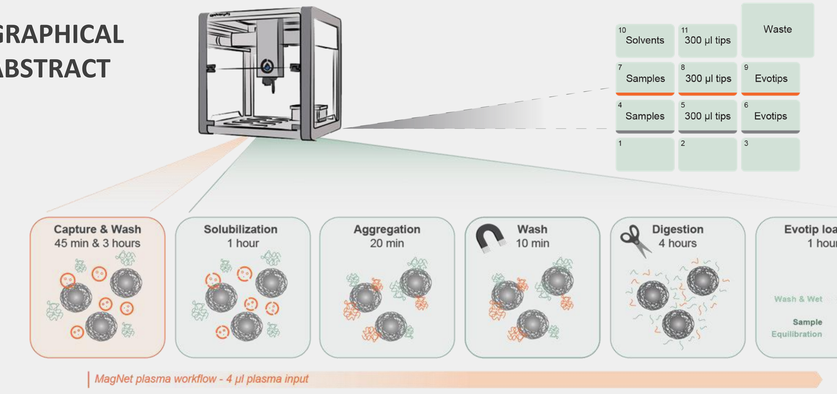
Inter-lab assessment of standardized and fully automated workflows integrated with Evosep One

Andrea Ellero[a], Camilla O. Kyhl[b], Joel M. Vej-Nielsen[b], Adele Nel[a], Anne Katrine Ravno[b], Ireshyn Govender[a], Previn Naicker[c], Stoyan Stoychev[a,b], Dorte Bekker-Jensen[b], Nicolai Bache[b], Justin Jordaan[a]

[a] *ReSyn Biosciences, Pretoria, South Africa;* [b] *Evosep Biosystems, Odense, Denmark,* [c] *CSIR, Pretoria, South Africa, E-mail: sstoychev@resynbio.com*

GRAPHICAL ABSTRACT

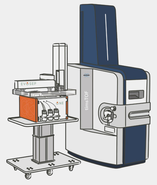


INTRODUCTION

- The ability to translate new leads to diagnostics and disease therapy is dependent on implementation of start-to-finish analytical workflows that can process clinical samples in a robust, high-throughput and cost-effective manner
- We assess the robustness of end-to-end analytical proteomics workflows, standardized across two independent laboratories
- Three distinct modular workflows were evaluated, digest preparation, neat plasma profiling, and deep plasma profiling using Mag-Net™
- Methods are implemented on Opentrons OT2 liquid handler and use MagReSyn® hyper-porous magnetic microparticles, with a current throughput of 192 samples processed in less than 8 hours, from cell lysate (or raw plasma) to digest loaded onto an Evotip.

METHODS

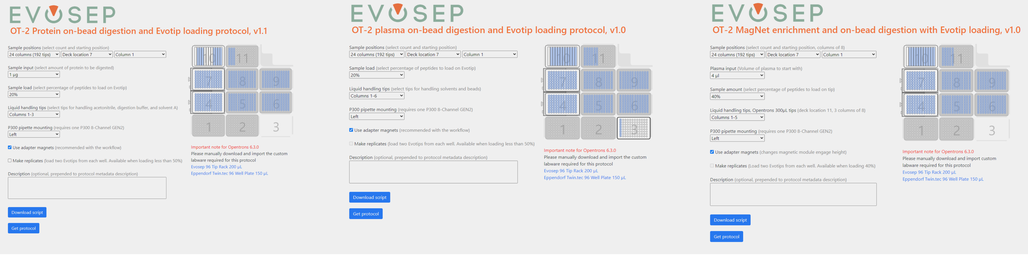
- HeLa cells were cultured and harvested at each site prior to extraction in single-pot buffer (5% SDS, 5mM TCEP, 10mM CAA)
- Single donor plasma for the neat and Mag-Net™ workflows was collected using EDRN SOP at each test site from consenting donors.
- Fully automated sample preparation was performed using Protein Aggregation Capture (PAC), for cell lysate or neat plasma, and Mag-Net™ for deep plasma profiling. All workflows were set up on an OT2 liquid handling system with integrated Evotip loading. LCMS analysis was standardized on an EvosepOne using 100SPD, coupled to Bruker TimsTOF HT in diaPASEF mode (default short gradient diaPASEF). Data extraction was performed with DIA-NN using default settings.
- *Study limitation: identical samples sources could not be obtained to verify each workflow to eliminate differences in performance due to biological variability of the plasma that was collected.



40%-80% digest was loaded on Evotip, analysis using Evosep One 100SPD, diaPASEF short gradient method on Bruker TIMS ToF HT

DIA-NN (version 1.8.1) in library-free mode. Conditions were searched separately.

STANDARDIZED ONLINE METHOD INTERFACE



OT2 Interface

Three end-to-end automated workflows were evaluated across two laboratory sites

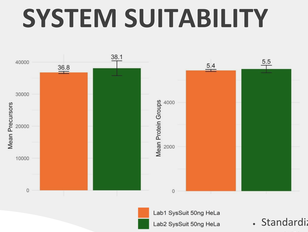
Standardized online OT2 scripting allows for users-friendly implementation of workflows

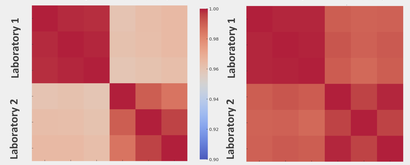
Evaluation of HeLa digestion showed high precision and reproducible digestion across sites

Robust Inter-laboratory analysis of clinically relevant plasma samples was shown with FDA approved biomarkers

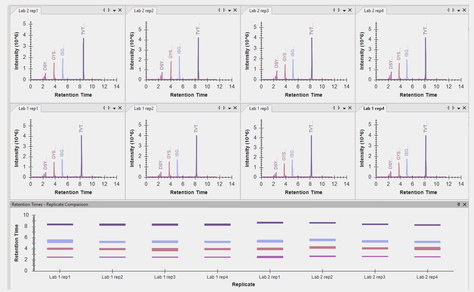
We demonstrate inter-laboratory deep plasma profiling using Mag-Net with only 4µl of plasma

SYSTEM SUITABILITY

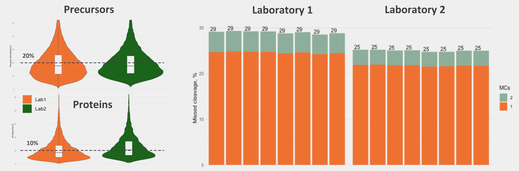




- Standardized 100 SPD Evosep One methods coupled to the robust timsTOF diaPASEF data acquisition enabled inter-laboratory comparison of the fully automated workflows.
- A total of 50ng Pierce HeLa digest was used as system suitability control, which showed highly reproducible performance across both laboratories with stable peptide retention times (**right**), and high correlation of quantified precursors and proteins (**above and above left**).



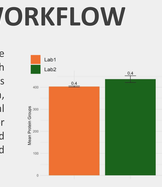
DIGEST PREPARATION

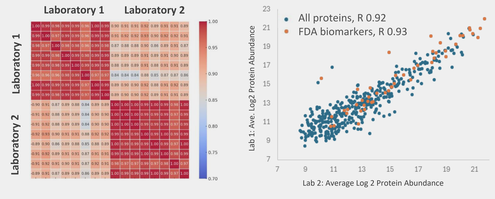


- Reproducible and robust digest preparation is the foundation of modular automated proteomics workflows, and in this instance uses Protein Aggregation Capture (Batth *et al.*, 2019, DOI:10.1074/mcp.TIR118.001270)
- 1µg HeLa extracts were independently processed across two laboratories using the fully automated OT2 workflow integrating all steps from raw lysate to Evotip loading of digests. The end-to-end method, enabled by the strong magnetic moment of MagReSyn® hydroxyl beads, performed reproducibly across each site with a variance of <4% for digestion efficiency.
- Coefficient of variance (CV) for precursors and Proteins (**far left**), and missed cleavages (**left**) detected across the two laboratories were comparable (n=16)

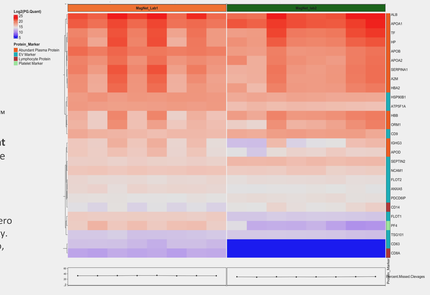
NEAT PLASMA WORKFLOW

- Approximately 400 plasma proteins were identified at each site (**right**) with high correlation across technical replicates (**middle right**): Pearson correlation, particularly considering biological variations due to different source of donor plasma used). Numerous FDA approved biomarkers could be reproducibly detected at each site (**far right**).

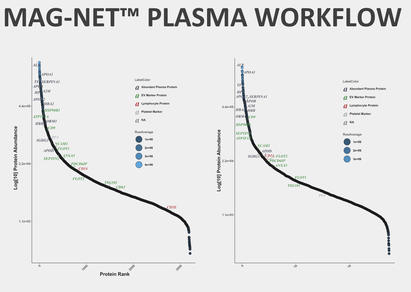




- Plasma (4µl) was processed using the fully automated Mag-Net™ OT2 workflow, which performed with high precision (CV above).
- Small differences in protein abundance (e.g. vesicle markers **right and far right**) may be attributed to biological variation at each site (different donors)



MAG-NET™ PLASMA WORKFLOW



CONFLICT OF INTEREST DISCLOSURE
Stoyan Stoychev, Justin Jordaan, Ireshyn Govender, Adele Nel, Andrea Ellero are employed by ReSyn Biosciences, proprietors of MagReSyn® technology. Stoyan Stoychev, Camilla O. Kyhl, Joel M. Vej-Nielsen, Anne Katrine Ravno, Dorte Bekker-Jensen and Nicolai Bache are employed by Evosep.