

Neat plasma workflow - 1 µl plasma input

Digest preparation workflow – 1  $\mu$ g HeLa input

## 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<sup>™</sup> 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<sup>™</sup> 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.

### **STANDARDIZED ONLINE METHOD INTERFACE**

# EVUSEP

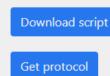
OT-2 Protein on-bead digestion and Evotip loading protocol, v1.1 Sample input (select amount of pro Sample load (select per Liquid handling tips (select tips for handling acetonitrile, digestion buffer, and solvent A) Columns 1-3 🗸 🗸 P300 pipette mounting (requires one P300 8-Channel GEN2) Left 🗸 ✓ Use adapter magnets (recommended with the workflow) Make replicates (load two Evotips from each well. Available when loading less than 50%)
labware required for this protocol Description (optional, prepended to protocol metadata description)

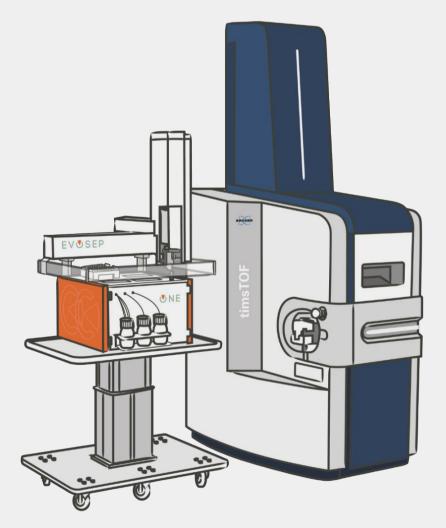
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	1	2	3					

Important note for Opentrons 6.3.0 Please manually download and import the custom Evosep 96 Tip Rack 200 µL Eppendorf Twin.tec 96 Well Plate 150 µL

### EVUSEP OT-2 plasma on-bead digestion and Evotip loading protocol, v1.0

24 columns (192 tips)	Deck location 7	*	Column 1 👻	10
Sample load (select perce	entage of peptides t	o load on	Evotip)	
20%	~			L 000000000000000000000000000000000000
iquid handling tips (sele	ect tips for handling :	solvents a	nd beads)	
Columns 1-6	~			
2300 pipette mounting (r Left	requires one P300 8-	Channel	GEN2)	
✓ Use adapter magnets (	(recommended with	the work	low)	1
Make replicates (load t	two Evotips from eac	ch well. Av	ailable when loading less that	
Description (optional, pre	epended to protocol	metadat	a description)	Important note fo Please manually o labware required Evosep 96 Tip Rad Eppendorf Twin.te
Download script				





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

Opentrons 6.3.0

or this protocol

96 Well Plate 150 µL

: 200 µL



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

### EVUSEP OT-2 MagNet enrichment and on-bead digestion with Evotip loading, v1.0

olumns (192 tips) V Deck location 7 V Column 1 Plasma input (Volume of plasma to start with 4 µl 🗸 🗸 tides to load on tip) 40% 🗸 Liquid handling tips, Opentrons 300µL tips (deck location 11, 3 columns of 8) Columns 1-5 🔹 👻 P300 pipette mounting (requires one P300 8-Channel GEN2) Left 🗸 Use adapter magnets (changes magnetic module engage height) ownload and import the custom Make replicates (Load two Evotips from each well. Available when loading 40%) Description (optional, prepended to protocol metadata description)

		9
		6
1	2	3

Important note for Opentrons 6.3.0 Please manually download and import custom labware required for this protocol Evosep 96 Tip Rack 200 µL Eppendorf Twin.tec 96 Well Plate 150 µL



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

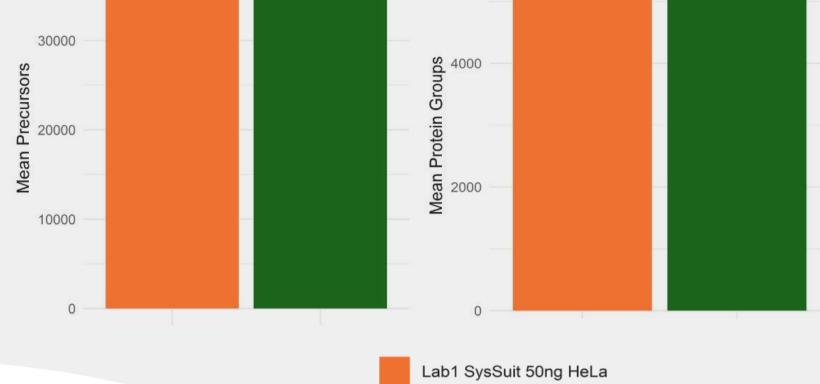
**Evaluation of HeLa digestion showed high** precision and reproducible digestion across sites

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

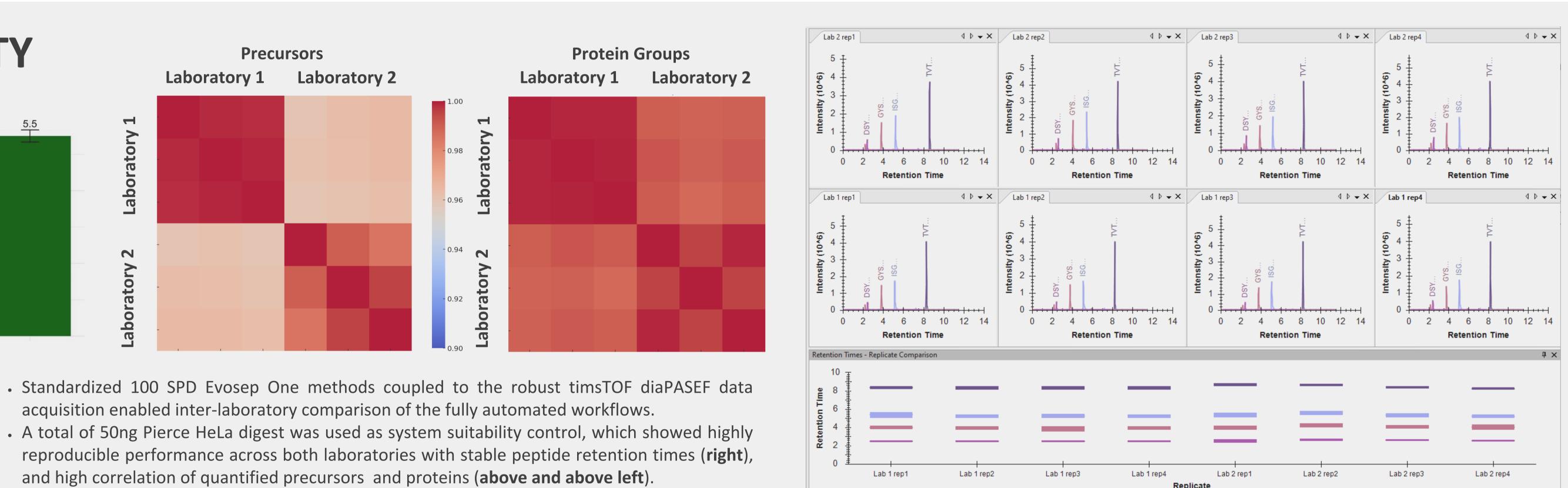
We demonstrate inter-laboratory deep plasma profiling using Mag-Net with only 4µl of plasma

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

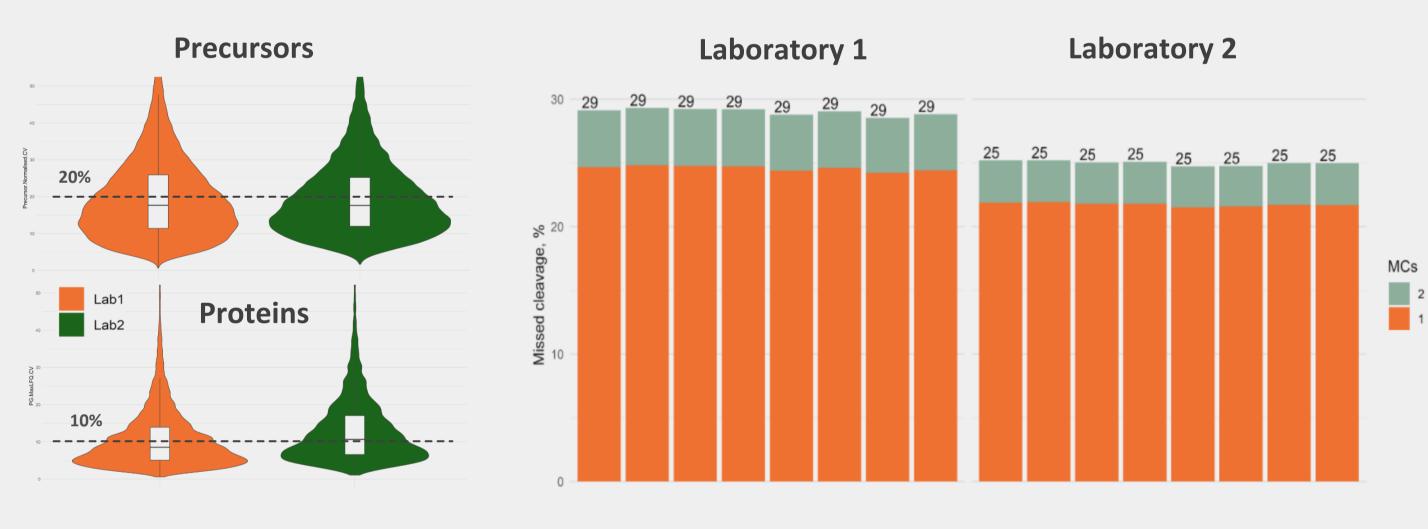
# **SYSTEM SUITABILITY**



Lab2 SysSuit 50ng HeLa

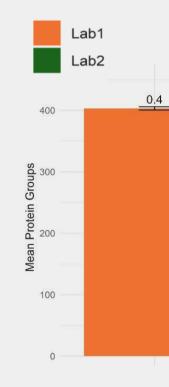


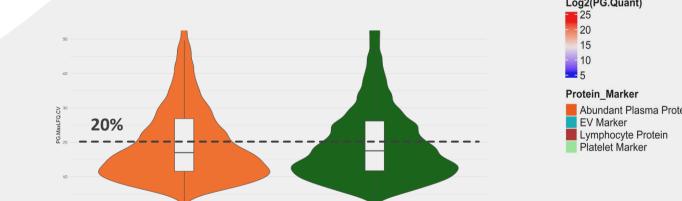
acquisition enabled inter-laboratory comparison of the fully automated workflows. and high correlation of quantified precursors and proteins (above and above left).



### **NEAT PLASMA WORKFLOW**

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

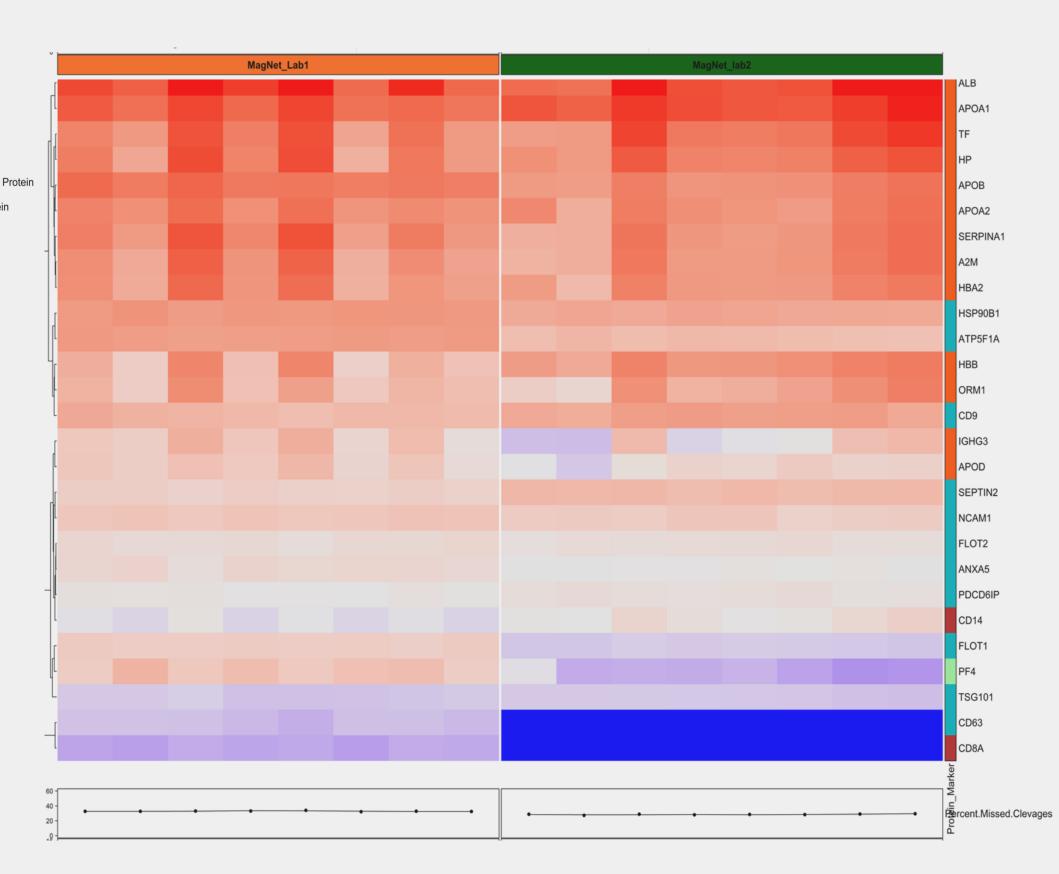




• Plasma (4µl) was processed using the fully automated Mag-Net<sup>™</sup> OT2 workflow, which performed with high precision (CV above). • Small differences in protein abundance (e.g. vesicle markers right and far right) may be attributed to biological variation at each site (different donors)

#### **CONFLICT OF INTEREST DISCLOSURE**

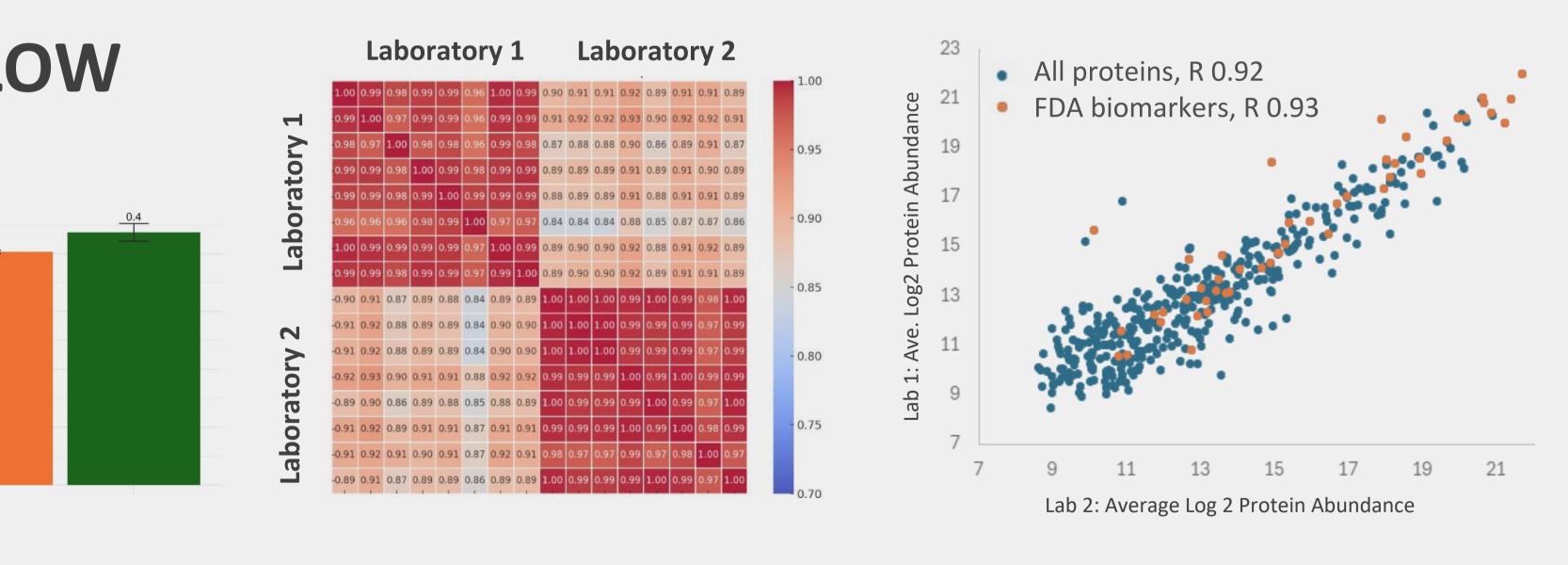
Stoyan Stoychev, Justin Jordaan, Ireshyn Govender, Adele Nel, Andrea Ellero are employed by ReSyn Biosciences, proprietors of MagReSyn® technology Stoyan Stoychev, Camilla O. Kyhl, Joel M. Vej-Nielsen, Anne Katrine Ravno, Dorte Bekker-Jensen and Nicolai Bache are employed by Evosep.



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### **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<sup>®</sup> 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)



### MAG-NET<sup>™</sup> PLASMA WORKFLOW

