AUTOMATED SAMPLE PREPARATION FOR BOTTOM-UP URINARY PROTEOME PROFILING IN CLINICAL PROTEOMICS

Ireshyn Govender[a,b], Previn Naicker[a], Andrea Ellero[c], Rethabile Mokoena[a,c], Stoyan Stoychev[b]

[a] Next Gen Health, CSIR, Pretoria, South Africa, [b] ReSyn Biosciences, Pretoria, South Africa, [c] University of Pretoria, Pretoria, South Africa, [d] University of the Witwatersrand, Johannesburg, E-mail: igovender@resynbio.com

GRAPHICAL ABSTRACT

COLLECTION OF CLINICAL URINE SAMPLES

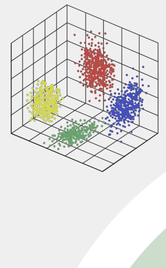
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

TSHEPONG HOSPITAL

RST PDAC iRSTM AKI + AKI -

NOVEL AUTOMATED URINE SAMPLE PROCESSING

DIFFERENTIATION IN CLINICAL COHORT



SIMPLER

HμLC: Direct on-bead urinary proteomics workflow: bind – wash – digest – LCMS

BETTER

Increased urinary proteome coverage with high reproducibility from low urine volume (100 µl)

FASTER

Rapid magnetic workflow without time consuming precipitation or centrifugation, ~2.5 min per sample

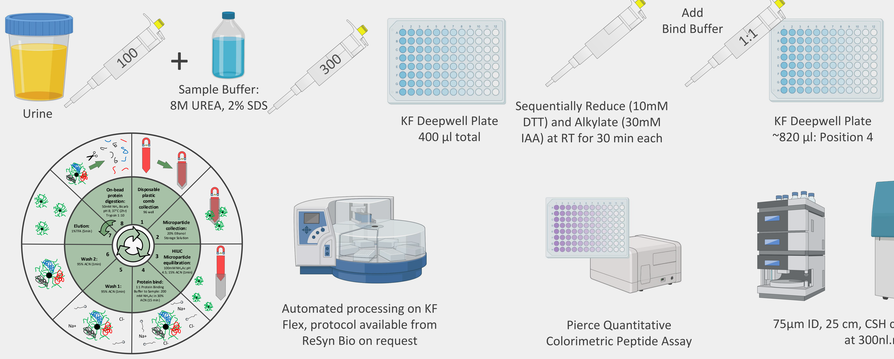
INTRODUCTION

- Urine is a diverse source of information related to a patient's health and is gradually becoming a go-to biofluid for clinical proteomics.
- There is currently no standard operating procedure for automated, reproducible & robust sample processing of urine for mass spec-based clinical proteomics
- Here, we present a novel workflow with direct protein capture on HILIC magnetic beads for Urine Low-volume Clean-up with on-bead digestion (HμLC), and compare this with a published on-membrane (OM) method developed by Berger *et al* (2015), as amended by Winter *et al* in 2021.
- The primary advantages of HμLC is that it only requires 100 µl of urine, with direct on-bead processing and full automation.
- The HμLC workflow was applied in two pilot studies: [A] Pancreatic Duct Adenocarcinoma (PDAC) & [B] Acute Kidney Injury (AKI)
- For Study A: Urine was collected from patients with resectable (RST) and irresectable or metastatic (iRST/M) PDAC at Chris Hani Baragwanath Hospital.
- Urine from AKI+/- patients was collected at the Tshepong Hospital Complex.
- We aimed to map the urinary proteomes of PDAC and AKI patients against a healthy cohort to gain clinical insights from their proteome profiles.

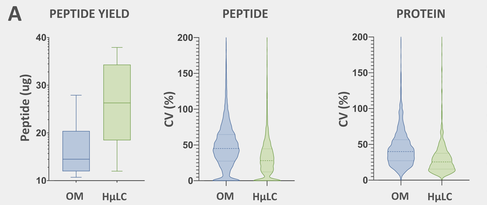
METHODS

- The OM (on-membrane) method was performed according to Winter *et al* (2021)
- The 2 workflows were compared using a 3 by 3 approach (n = 9 tech replicates). Peptides were analysed using an Ultimate 3000 coupled to a TripleTOF 5600.
- The HμLC workflow was applied in two distinct pilot studies (AKI and PDAC) to evaluate variations in the protein profiles between the cohorts.
- Data was searched using a study specific library in Spectronaut 17 and differentially abundant proteins (DAPs) were analysed using GO annotation software.

HULC WORKFLOW: LIQUID HANDLING

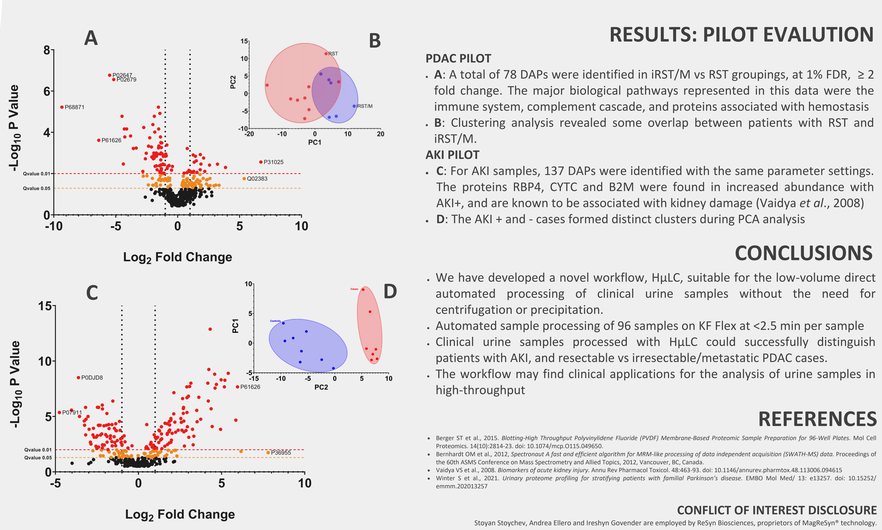


- *Instrument, plate and sample images courtesy of biorender.com*



RESULTS: BENCHMARKING OF WORKFLOW

- **A**: The HμLC method showed greater total peptide yield and lower CV's for peptide and protein groups than the OM method.
- **B**: There was a large overlap between the two methods as illustrated, with 7711 and 7477 peptides identified in the HμLC and OM workflows respectively, corresponding to 1140 and 1069 protein identifications
- **C**: Minor differences were observed in MW, pI and GRAVY score for the unique proteins identified in each workflow
- **D**: PCA plot showed that samples prepared using OM had a greater spread of data, indicative of poorer reproducibility between the technical replicates, while results from samples prepared with HμLC were more tightly clustered.
- **E**: The HμLC method appeared to identify a higher proportion of lower abundant proteins (16% vs 12% for OM)



RESULTS: PILOT EVALUATION

PDAC PILOT

- **A**: A total of 78 DAPs were identified in iRST/M vs RST groupings, at 1% FDR, ≥ 2 fold change. The major biological pathways represented in this data were the immune system, complement cascade, and proteins associated with hemostasis
- **B**: Clustering analysis revealed some overlap between patients with RST and iRST/M.

AKI PILOT

- **C**: For AKI samples, 137 DAPs were identified with the same parameter settings. The proteins RBP4, CYTC and B2M were found in increased abundance with AKI+, and are known to be associated with kidney damage (Vaidya *et al*., 2008)
- **D**: The AKI + and - cases formed distinct clusters during PCA analysis

CONCLUSIONS

- We have developed a novel workflow, HμLC, suitable for the low-volume direct automated processing of clinical urine samples without the need for centrifugation or precipitation.
- Automated sample processing of 96 samples on KF Flex at <2.5 min per sample
- Clinical urine samples processed with HμLC could successfully distinguish patients with AKI, and resectable vs irresectable/metastatic PDAC cases.
- The workflow may find clinical applications for the analysis of urine samples in high-throughput

REFERENCES

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CONFLICT OF INTEREST DISCLOSURE

Stoyan Stoychev, Andrea Ellero and Ireshyn Govender are employed by ReSyn Biosciences, proprietors of MagReSyn® technology.