Poster Reprint

ASMS 2021
Poster number MP005

Case Study of Inter-lab Cross-platform Transfer of a Protein Biomarker Quantitation Assay for Routine Analysis in Clinical Research

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Introduction

Diabetes is the leading cause of kidney failure. Current clinical tests to monitor Diabetic Kidney Disease (DKD) have limited power to predict early DKD. Therefore, it is meaningful to identify new protein biomarkers and develop tests for early prediction of DKD.

An MRM-based clinical research test , called PromarkerD, has been developed to predict kidney disease in diabetes.[1,2] The initial method developed at Proteomics International, Australia involved measurement of four proteins in immunodepleted plasma using a nanoflow LC/MS.[1,2] Since standard flow-based LC/MS is more appropriate for routine analysis the PromarkerD test was transferred to standard flow-based Agilent triple quadrupole (TQ) LC/MS system at Atturos, Dublin.[3]

The data sets acquired on both platforms were compared. In addition, the analytical quality for PromarkerD on standard flow 6495 LC/TQ was also compared in whole plasma and in immunodepleted plasma.

Experimental

Instrumentation

Agilent 1290 Infinity II LC system coupled to a 6495 Triple Quadrupole LC/MS (G6495B) with Jet Stream Technology Ion Source (AJS).

Plasma samples

All subjects' plasma samples were provided by the Fremantle Diabetes Study (FDS), a longitudinal observational cohort. A standard reference plasma was collected from three healthy volunteers and combined before aliquoting and storage at –80 °C.

Method transfer

To establish the PromarkerD method on the Agilent 6495 LC/TQ at Atturos, a synthetic peptide mix containing peptides representative of potential DKD protein biomarkers was generated and spiked into Atturos reference serum (ARS) for analysis of PromarkerD.

LC/MS analysis

All samples were separated using the Agilent ZORBAX Eclipse Plus Rapid Resolution C18 analytical column: 50 × 2.1 mm, 1.8 µm in size (p/n 959757-902).

Buffer A consisted of 99.9% H_2O with 0.1% formic acid and buffer B consisted of 99.9% acetonitrile with 0.1% formic acid. Data was acquired in dynamic MRM mode (Table 1). Data analysis for targeted peptide quantification was carried out using Skyline software.

Experimental

Agilent 6495 LC/TQ	
Ion Mode	AJS ESI, positive mode
Gas Temperature	150 °C
Drying Gas Flow	15 L/min
Capillary Voltage	4000 V
Nozzle Voltage	300 V
High/Low Pressure RF	150/60 V
Delta EMV	300 V
MS1 / MS2 Resolution	Unit / Unit
Cycle Time	500 ms

Agilent 1290 Infinity II LC			
Time (minutes)	A (%)	B (%)	Flow (mL/min)
2	92	8	0.400
18	70	30	0.400
22	10	90	0.400
25	10	90	0.400
25.10	97	3	0.400
26	97	3	0.400

Table 1: Overview of LC/TQ acquisition parameters.

Results and Discussion.

PromarkerD protein biomarkers

During development of PromarkerD method, the list of protein biomarkers was cut down to four proteins when this study was carried out. The targeted 8 peptides from the four protein biomarkers are listed in Table 2.

Uniprot ID	Protein name	Targeted peptides
APOA4_HUMAN	Apolipoprotein A-IV	LEPYADQLR
		ISASAEELR
CD5L_HUMAN	CD5 antigen-like	LVGGDNLCSGR
		IWLDNVR
FHR2_HUMAN	Complement factor H-related protein 2	TGDIVEFVCK
		LVYPSCEEK
IBP3_HUMAN	Insulin-like growth factor-binding protein 3	ALAQCAPPPAVCAELVR
		FLNVLSPR

Table 2: Targeted proteins and peptides.

Nanoflow to standard flow comparison

To assess the measurement comparability of the PromarkerD biomarkers on nanoflow and standard flow LC/TQ platforms, 12 plasma samples (PI-4205 A to L) were prepared by Proteomics International (PI) and then sent to Atturos.

- At PI, samples were analyzed on a non-Agilent nanoflow LC/MS platform
- At Atturos, samples were analyzed on an Agilent standard flow 6495 LC/TQ
- Overall, the data obtained on both platforms are very comparable (Figure 1)

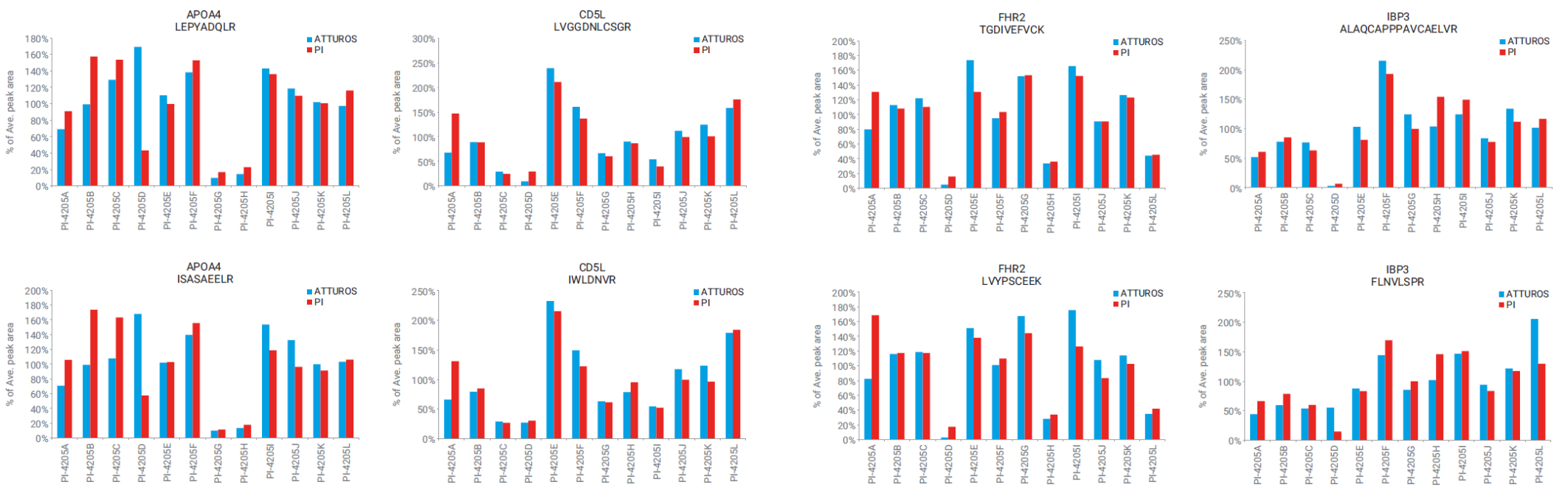

Figure 1. Comparison of eight peptides from the four selected proteins across 12 plasma samples analyzed by nanoflow LC/MS in PI (red bars) and standard flow LC/TQ in Atturos (blue bars). The individual subject's peak area for a given peptide is expressed as a percentage of the average peak area for that peptide across the 12 plasma samples.

MRM chromatograms of LEPYADQLR peptide representing APOA4

Analysis of MRM chromatograms for LEPYADQLR peptide from APOA4 using standard flow 6495 LC/TQ proved symmetrical peaks with high intensity and low background (Figure 2A). The high dot-product (dotp) values for the peak in each sample confirmed the similarity of relative product ion distribution in the real samples and in the library (Figure 2B). This indicates high quality of targeted quantitation on this platform. Therefore, the occasional deviation between the two data sets might be due to variation in sample handling.

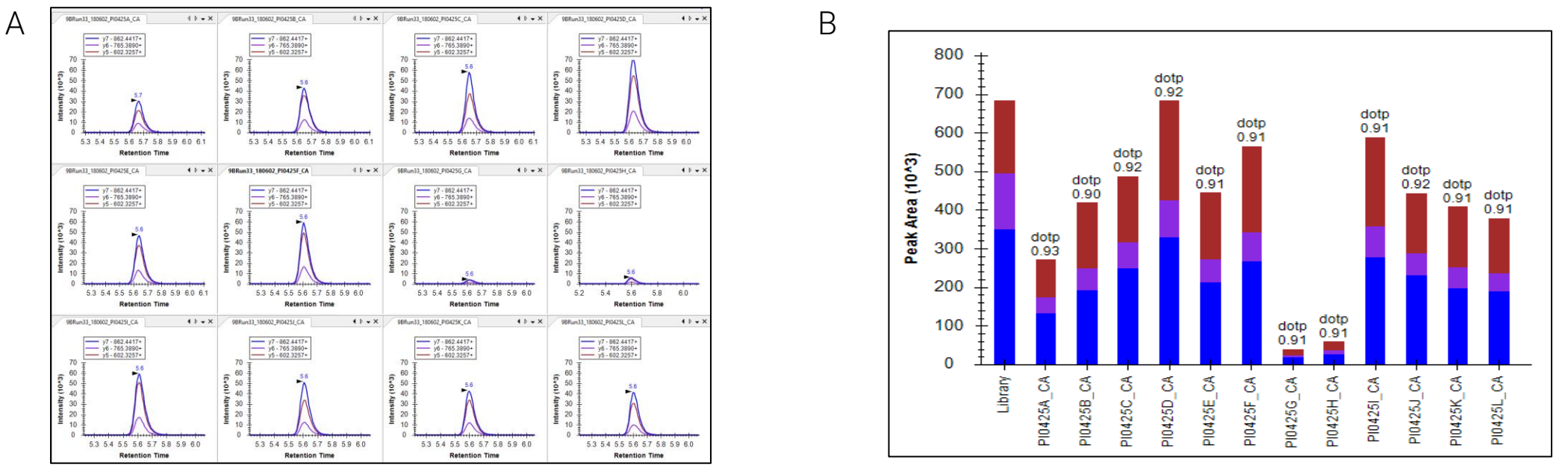

Figure 2. Peak area of the LEPYADQLR peptide from APOA4 across the 12 plasma samples. A. MRM chromatograms of LEPYADQLR peptide in 12 plasma samples. B. Stacked bar chart of peak areas of the targeted transitions displayed with dot-product (dotp) value between the peak areas and the matching MS/MS peak intensities.

Results and Discussion

Depleted plasma versus whole plasma on the 6495 LC/TQ

The PromarkerD biomarkers were measured on the Agilent 6495 LC/TQ in whole reference plasma and reference plasma depleted of the top 14 serum proteins using the Agilent Multiple Affinity Removal Column Human 14. The MRM chromatograms of all eight peptides are shown in Figure 3.

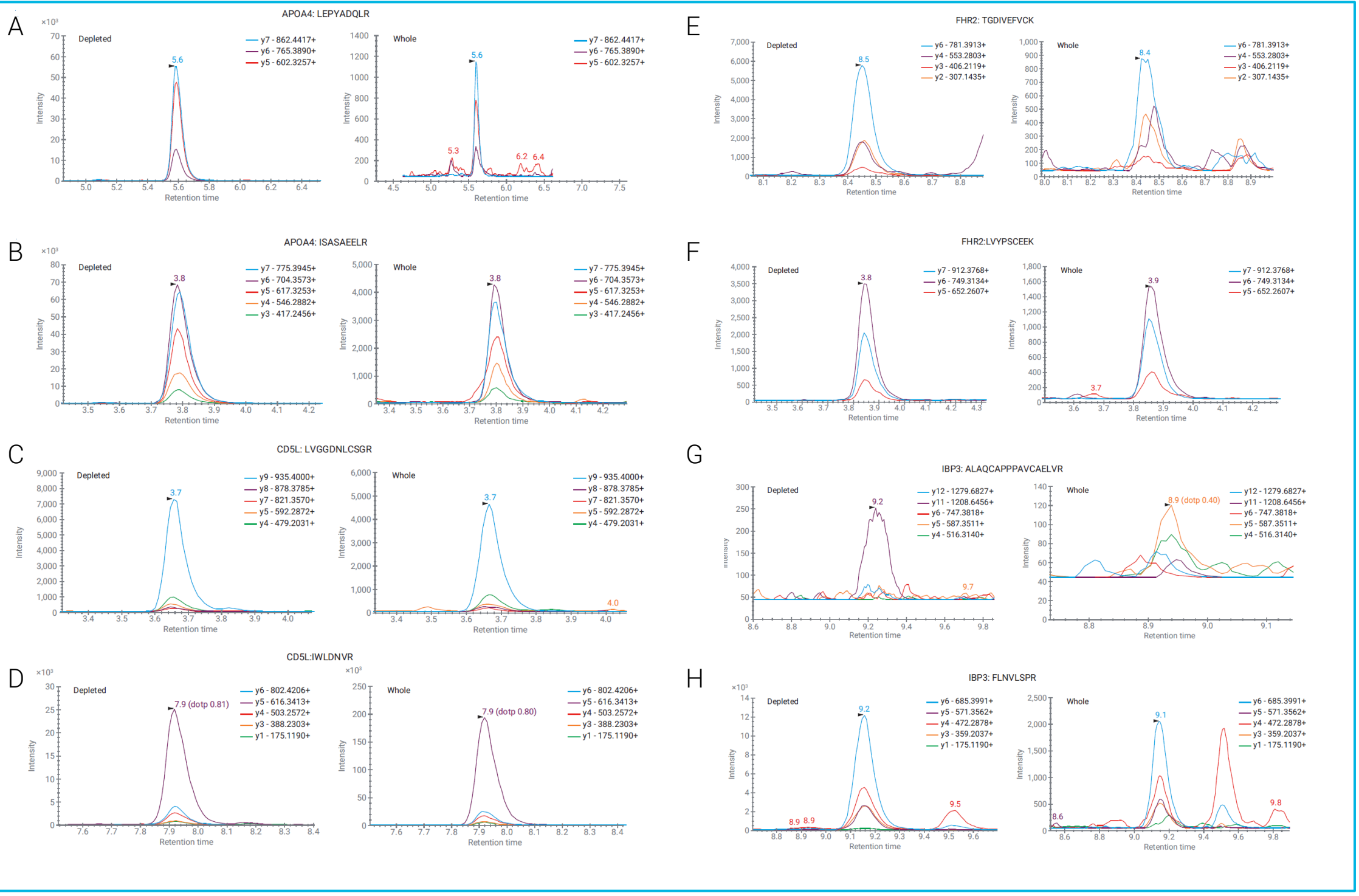

Figure 3. MRM chromatograms for all eight peptides (A-H) targeting four proteins (APOA4, CD5L, FHR2, and IBP3) in depleted and whole reference plasma.

Conclusions

This study demonstrated a successful inter-lab and cross-platform method transfer from nanoflow to standard flow LC/MS:

- Standard flow-based Agilent 6495 LC/TQ provides excellent quantification performance in complex matrices.
- The analytical sensitivity of 6495 LC/TQ allowed for detection of the four PromarkerD proteins in whole plasma without depletion.
- The workflow established at Atturos is more appropriate for routine analysis and should help facilitate the transfer of PromarkerD into clinical use.

References

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[3]Coleman, O. *et al.* From Nanoflow to Standard Flow LC/MS for Routine Quantitative Plasma Proteomics in Diabetic Kidney Disease Research. *Agilent application note*, publication number 5994-2381EN, 2020

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RA44474.3249884259

Published in USA, October 20, 2021

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