PromarkerD as an immunoaffinity mass spectrometry assay for diabetic kidney disease

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www.PromarkerD.com

PromarkerD

- **PromarkerD is a novel test for predicting diabetic kidney disease (DKD).**
- **The PromarkerD risk score is based on 3 plasma protein biomarker concentrations (CD5L, APOA4, and IGFBP3) together with 3 clinical metrics (age, HDL-cholesterol, and eGFR)[1,2].**
- **The immunodepletion method originally developed was converted and optimised into an immunoaffinity-based mass spectrometry (IA-MS) assay.**
- **In a 100-person cohort, the 3 plasma biomarkers were measured using both the immunodepletion and IA-MS assays. The PromarkerD risk scores were calculated for each set of data.**
- **The same cohort was independently analysed by a second laboratory (Atturos) using the IA-MS assay and the PromarkerD risk scores compared between laboratories.**

PromarkerD Workflow

Immunodepletion

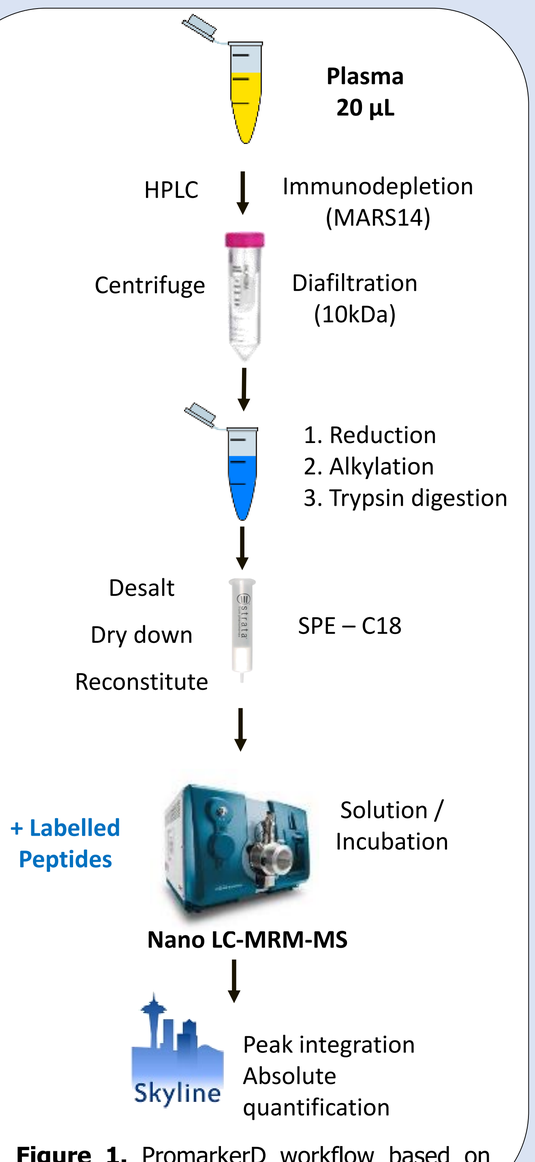

Figure 1. PromarkerD workflow based on the immunodepletion method.

Immunoaffinity-Mass Spectrometry

METHOD

ANTIBODY-BEAD PREPARATION
- Antibodies were produced for each of the 3 biomarkers.
- Antibodies were coupled to magnetic beads and purified.
- Ab-Beads for each biomarker were pooled.

PLASMA & ANTIBODY-BEAD
- Transfer of antibody-bead solution and plasma into 96-well plate.
- Includes calibrators and QC.
- Incubate at 37°C with mixing.

PROCESSING
- Removal of incubation liquid and 2x washes with buffer.
- Reduction and alkylation on bead.
- Add labelled synthetic biomarker peptides (for normalisation of signal).
- Trypsin digest O/N, 37°C, mixing.

LC-MRM-MS
- Microflow LC coupled with Sciex 5500 (Microflow has advantage of robustness, throughput, vs nanoflow. Sensitivity increased compared to typical analytical flow).

ANALYSIS
- Peak integration and absolute quantification of 3 biomarkers

Figure 2. PromarkerD workflow based on the immunoaffinity bead method.

Cross-platform validation

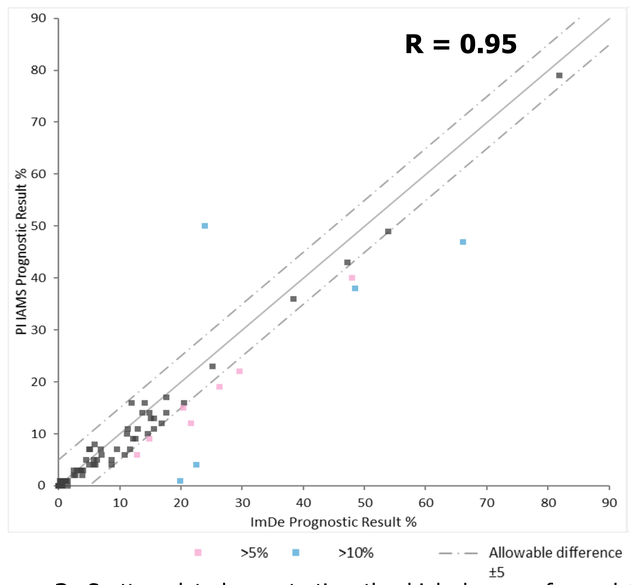

Figure 3. Scatter plot demonstrating the high-degree of correlation between the immunodepletion method and the IA-MS method. Generated after individual biomarker Bland-Altman analyses between the methods.

Cross-centre validation

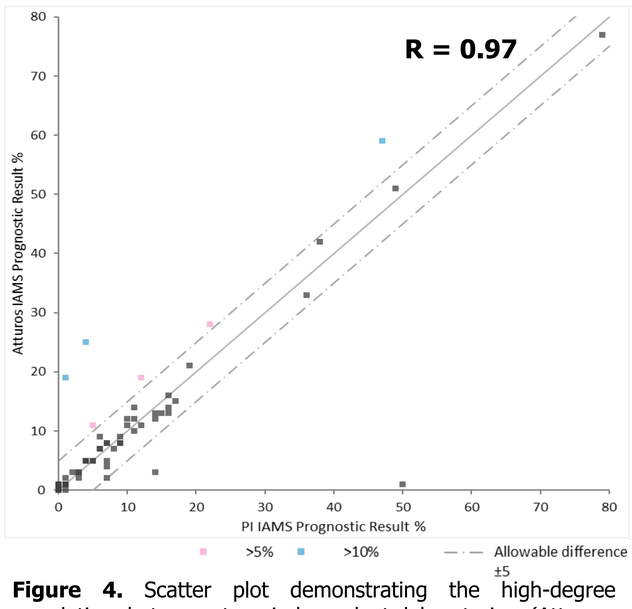

Figure 4. Scatter plot demonstrating the high-degree of correlation between two independent laboratories (Atturos and Proteomics International) with the IA-MS method (after individual biomarker Bland-Altman analyses between the laboratories. Atturos platform includes Agilent LC and 6495B MS.

Stability

Protein	4°C		RT		24 h		Freeze-thaw		Extract
	1 h vs 24 h (N=3)		1 h vs 24 h (N=3)		RT vs 4°C (N=3)		3 vs 1 freeze-thaw (N=3)		24 h, 4°C, N=86, autosampler
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Precision
APOA4	101.6	10.0	92.6	7.4	84.0	3.6	100.8	19.0	0.9
CD5L	118.4	8.6	103.8	10.1	95.4	14.5	106.1	14.8	8.5
IBP3	95.3	13.9	91.8	5.5	99.6	4.3	95.2	15.1	2.1

Accuracy and precision data are expressed as percentage. Precision based on the average percent coefficient of variation (CV). Stability testing acceptance criteria were accuracy <20% CV deviation from the control value and precision of <20% CV.

Accuracy and Precision

Protein	Intra-day (N=4)		Inter-day (N=20)	
	Concentration (µg/mL)	Precision (%)	Concentration (µg/mL)	Precision (%)
APOA4	79.4 ± 7.5	9.4	75.8 ± 7.1	9.4
CD5L	2.77 ± 0.21	7.6	2.50 ± 0.24	9.8
IGFBP3	0.27 ± 0.01	5.6	0.29 ± 0.03	10.5

Data are mean ± SD. Concentration data represent concentrations found with the PromarkerD assay. Precision based on the average percent coefficient of variation (CV). Intra- and inter-assay precision acceptance is <20% CV.

Summary

The IA-MS platform developed for PromarkerD confers additional advantages compared with the immunodepletion method.

Features of the new IA-MS method includes (a) increased throughput with a 96-well plate format coupled with a robotic handling system, (b) cleaner sample preparation enhancing sensitivity and reducing instrument down-time, (c) microflow LC maintains sensitivity (vs nanoflow) with increased robustness, and (d) simplifies technology transfer processes to partner with LCMS capability.

The IA-MS method is platform-independent, showing a high-degree of correlation between two independent laboratories as well as between the two methods.

References: (1) EuPA Open Proteomics (2017) 14: 1-10 (2) Diabetes Care (2017) 40: 1548. **Acknowledgements**: The 100-person cohort was sourced from the Fremantle Diabetes Study Phase II (NHMRC grants #513781 and #1042231). The MS analyses were performed in facilities provided by the LotteryWest State Biomedical Facility-Proteomics node and Bioplatforms Australia at the Harry Perkins Institute for Medical Research.