ASX Release
10 February 2020

ASX code: PIQ

PromarkerD presented at Australasian Proteomics conference

Proteomics International Laboratories Ltd (Proteomics International; ASX: PIQ) announces the attached presentation on PromarkerD (MS)[#] was given yesterday at the 25th Annual Lorne Proteomics Symposium, Lorne Victoria 6-9 February 2020, Australasia's premier conference on proteomics.

Proteomics International's Research Manager, Dr Scott Bringans was invited to speak in the New Technologies session on the company's pioneering test for predicting the onset of diabetic kidney disease.

The presentation described the successful cross-validation of PromarkerD (MS) with the company's partner, clinical diagnostics firm Atturos, Ireland [ASX: 12 September 2019], along with the technical developments that underpin the test, which recently achieved CE Mark registration in Europe [ASX: 12 November 2019 and 14 January 2020].

Definitions:
"Promarker" - the proprietary technology used to discover and evaluate proteins for use as diagnostics
"PromarkerD/PromarkerD test system" - the patented predictive diagnostic test for Diabetic Kidney Disease
"PromarkerD (MS)" - the predictive diagnostic test for Diabetic Kidney Disease using Mass Spectrometry
"PromarkerD (IA)" - the predictive diagnostic test for Diabetic Kidney Disease using ImmunoAssay
"PromarkerD Hub" - the proprietary software tool used to calculate the risk of Diabetic Kidney Disease in diabetes patients

ENDS

About PromarkerD (www.PromarkerD.com)
The PromarkerD test system assesses the risk of diabetic kidney disease (DKD) in patients with type 2 diabetes. Chronic kidney disease is one of the major complications arising from diabetes and if unchecked can lead to dialysis or kidney transplant. PromarkerD is a simple blood test that uses a unique protein 'fingerprint' to provide an early detection of the onset of disease. In clinical studies published in leading journals PromarkerD correctly predicted 86% of otherwise healthy diabetics who went on to develop chronic kidney disease within four years.

Further information is available through the PromarkerD web portal.

About Proteomics International Laboratories (PILL) (www.proteomicsinternational.com)
Proteomics International (Perth, Western Australia) is a wholly owned subsidiary and trading name of PILL (ASX: PIQ), a medical technology company at the forefront of predictive diagnostics and bio-analytical services. The Company specialises in the area of proteomics – the industrial scale study of the structure and function of proteins. It received the world's first ISO 17025 laboratory accreditation for proteomics services, and operates from state-of-the-art facilities located on Perth's QEII Medical Campus.

Proteomics International's business model is centred on the commercialisation of the Company's world-leading test for diabetic kidney disease, PromarkerD. The Company offsets the cash burn from

R&D and product development through provision of specialist analytical services, whilst using its proprietary Promarker™ technology platform to create a pipeline of novel diagnostic tests.

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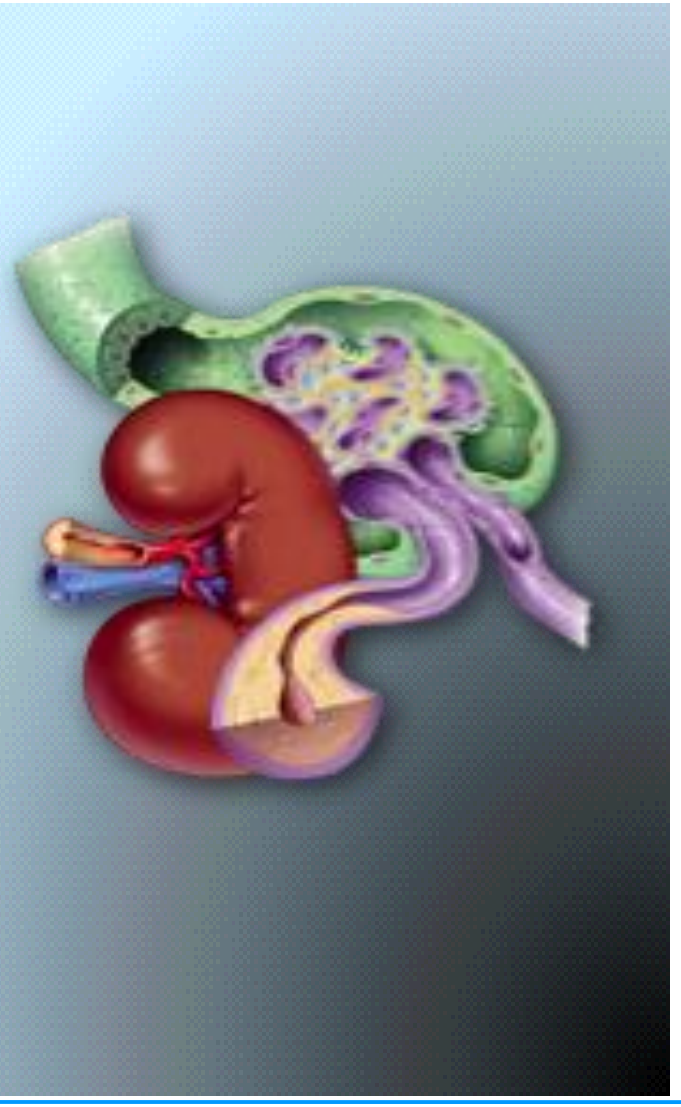
Proteomics International

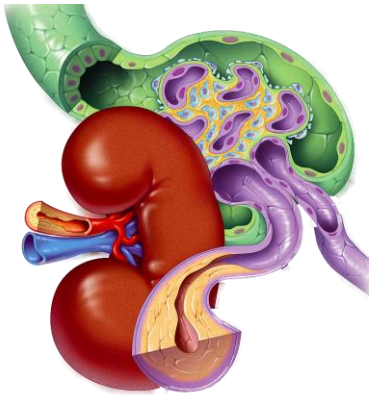
PromarkerD as an immunoaffinity mass spectrometry assay for diabetic kidney disease

Scott Bringans PhD
Research Manager, Proteomics International



Outline

- Diabetic Kidney Disease
- PromarkerD Test
- Original Immunodepletion Method
- Immunoaffinity Mass Spectrometry
- Reproducibility and Precision
- Cross-Platform Validation
- Cross-Centre Validation
- Conclusions



Diabetic Kidney Disease

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- Complication of diabetes
- 1 in 3 adult diabetics currently have DKD
- Current tests
  - ACR – urinary albumin:creatinine ratio
  - eGFR – estimated glomerular filtration rate

PromarkerD Test

Proteomics International

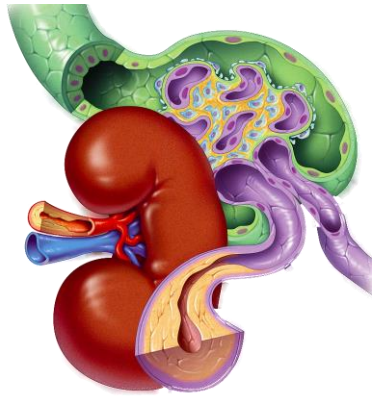
- PromarkerD is a novel test for **predicting** diabetic kidney disease (DKD).

- The PromarkerD risk score comprises
  - 3 plasma protein biomarker concentrations
    CD5L APOA4 IGFBP3
  - 3 clinical metrics
    age HDL-cholesterol eGFR

PromarkerD
CHANGING LIVES

Prognostic Biomarkers

eGFR decliners – can the biomarkers predict who will develop diabetic kidney disease?



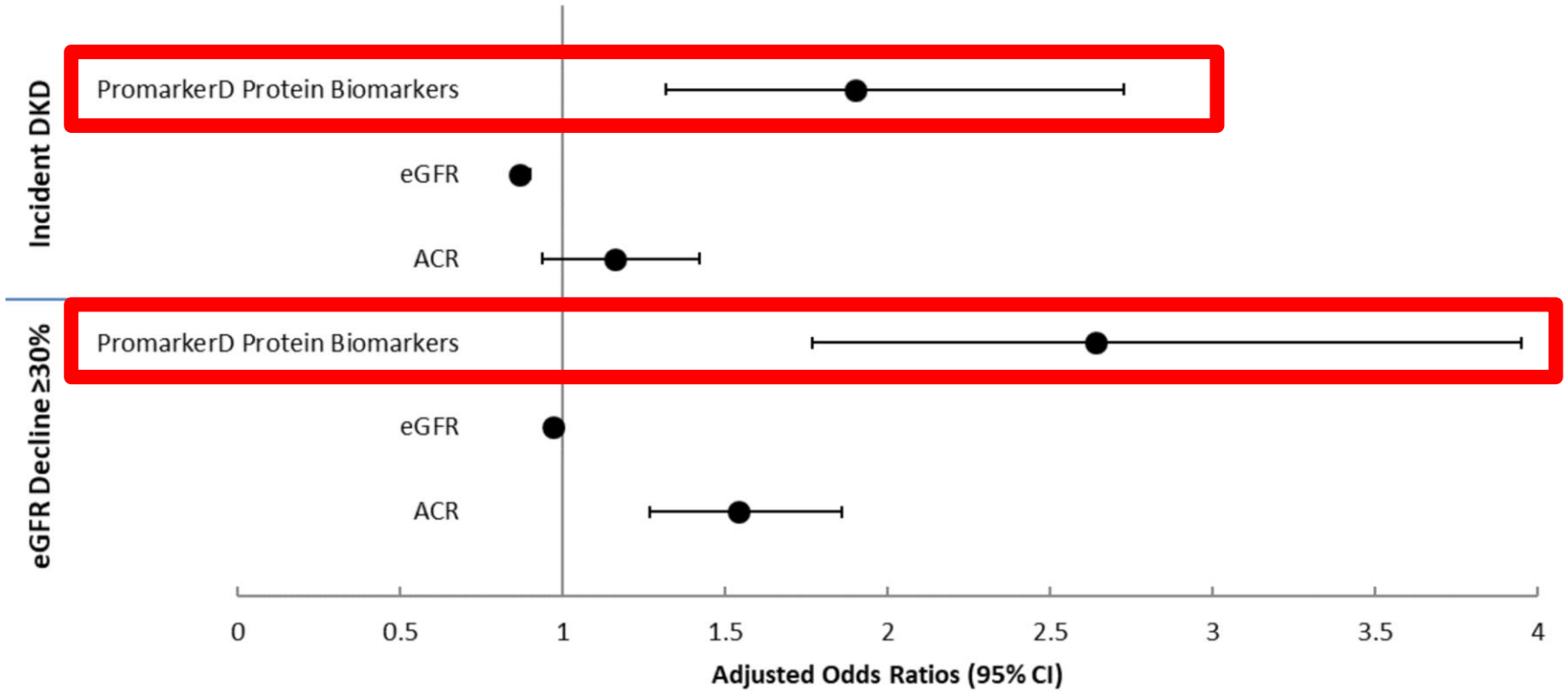
- A rapidly declining eGFR is one of the strongest indicators of significant renal impairment and a steady progression of diabetic kidney disease
- PromarkerD **predicts** rapid eGFR decline in type 2 diabetes across clinically significant definitions of diabetic kidney disease independently of recognised clinical risk factors.

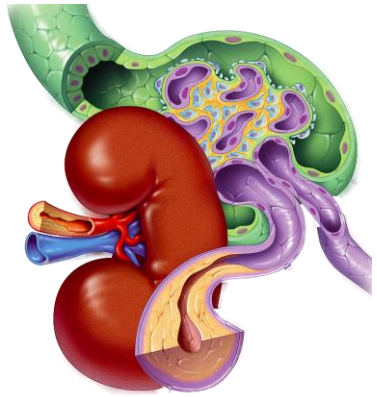
PromarkerD can correctly predict 86% of the previously kidney disease-free diabetic patients who go on to develop chronic kidney disease.

Sensitivity 86%, Specificity 78%, AUC 0.88

J. Diabetes and its Complications (2019)

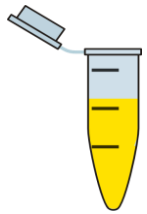
Prognostic Biomarkers





- Adjusted odds ratio of biomarkers for predicting renal decline.
- Odds ratios are used to express the relative chance of an event happening under two different conditions

PromarkerD (MS) Immunodepletion



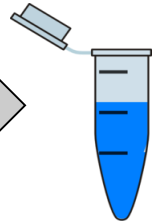
Plasma 20uL

Immunodepletion (MARS14)

HPLC

Diafiltration (10kDa)

Centrifuge



Digest

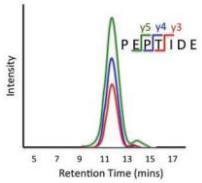
SPE – C18

Clean-up

+ spike labelled peptides

Analyse

Nano LC-MRM-MS
300nL/min
20 min run

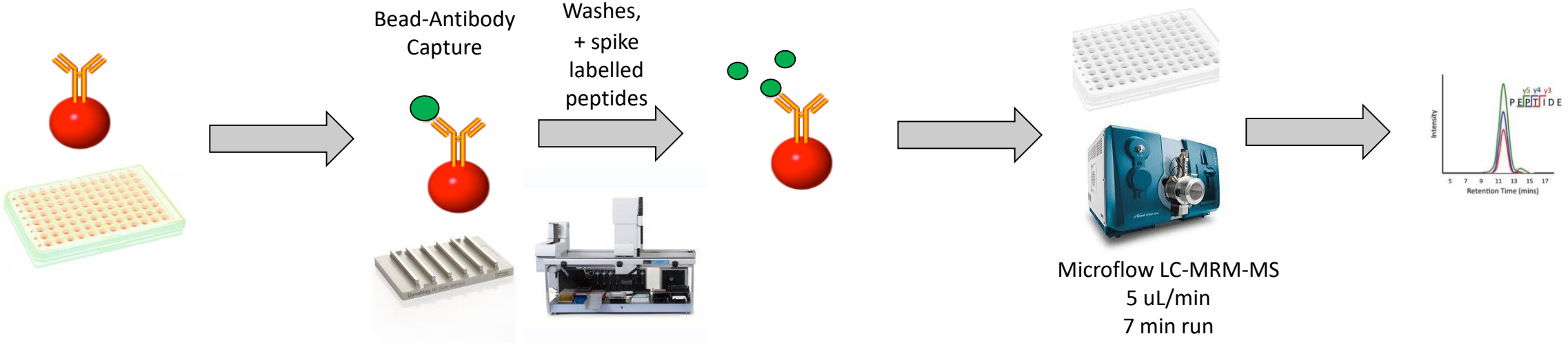


Quantify

1 week
Labour intensive
Expensive
But it works!

EuPA Open Proteomics (2017) 14: 1-10

PromarkerD (MS) Immunoaffinity

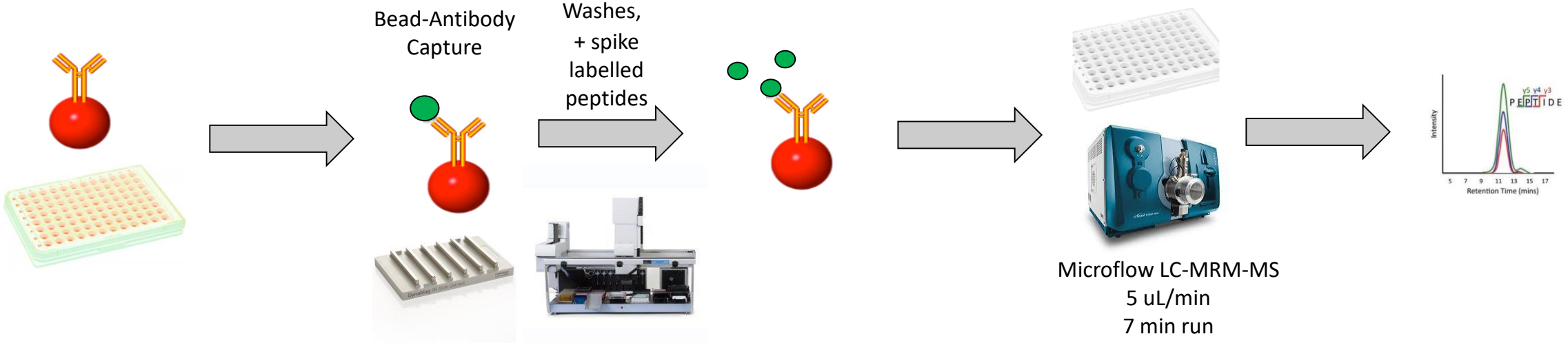


ANTIBODY-BEAD PREPARATION

- Antibodies produced for each of the 3 biomarkers.
- Antibodies coupled to magnetic beads and purified.
- Ab-Beads for 3 biomarkers pooled.

2 days

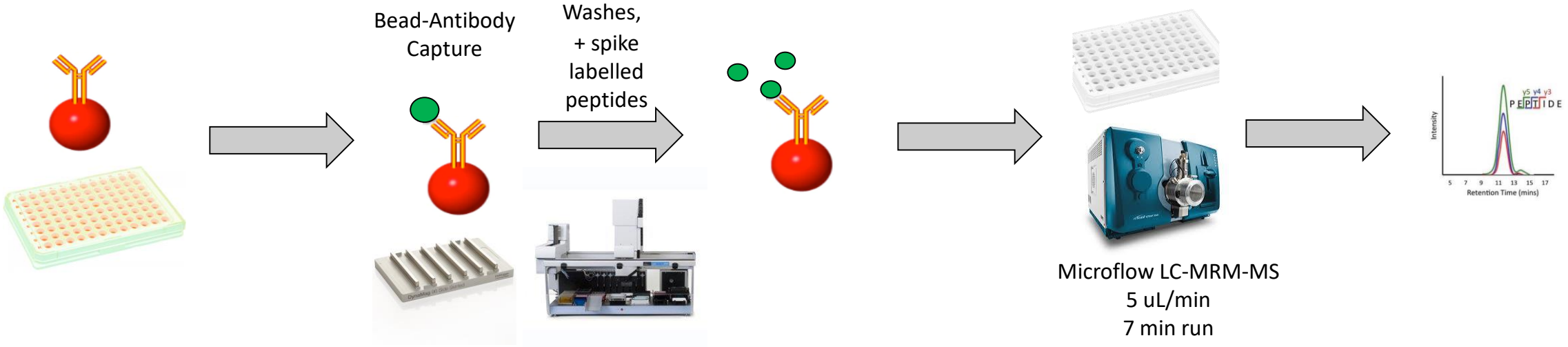
PromarkerD (MS) Immunoaffinity



PLASMA & ANTIBODY-BEAD

- Transfer of antibody-bead solution and plasma into 96-well plate. 2 days
- Includes calibrators, QCs and blanks.
- Incubate at 37°C with mixing.

PromarkerD (MS) Immunoaffinity

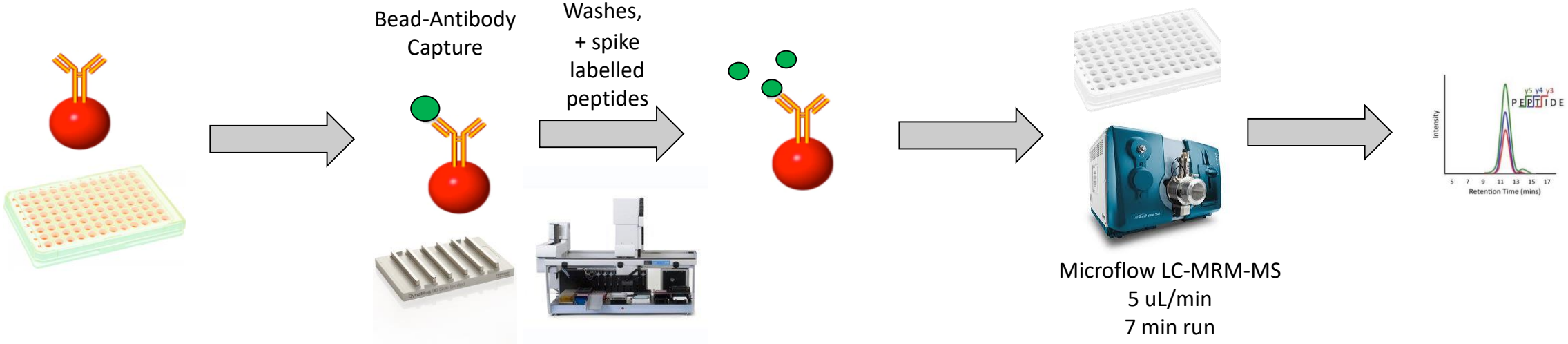


PROCESSING

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

2 days

PromarkerD (MS) Immunoaffinity

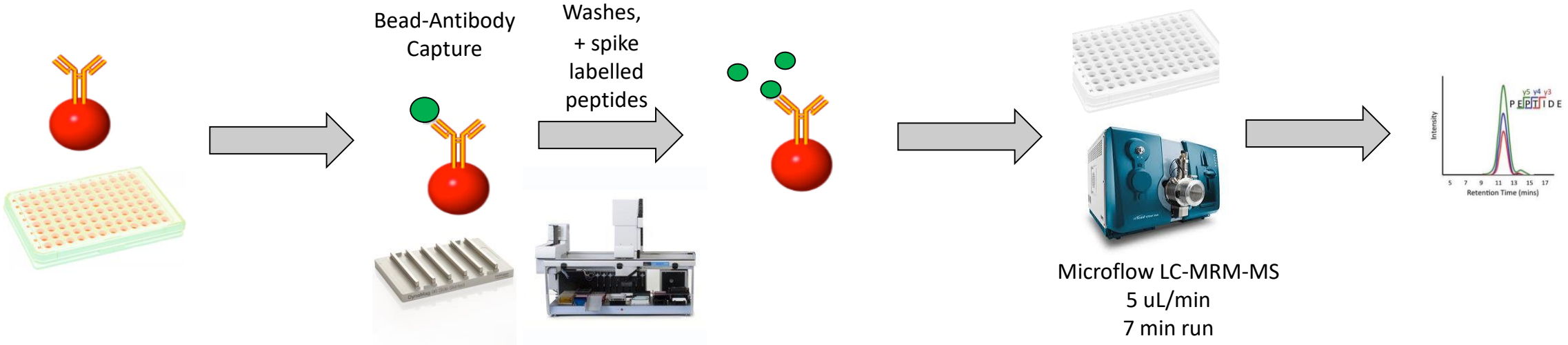


LC-MRM-MS

- Microflow LC coupled with Sciex 5500
- 5 uL/min
- 7 min run

2 days

PromarkerD (MS) Immunoaffinity



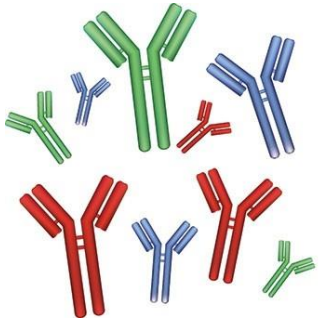
2 days

ANALYSIS

- Peak integration and absolute quantification of 3 biomarkers

Immunoaffinity Advantages

- Selectivity - just what you need
- Time - automation, less hands on time
- Cleanliness - no dirty background plasma
- Microflow - approach sensitivity of nanoflow
  - load more than on nano columns
  - far superior to analytical flows
  - robust source, spray stability
- Tech transfer - easier method, easier to copy



Cons

- Only detect proteins if you have an antibody

Reproducibility and Precision

Proteomics International

Reference plasma.
Equivalent to biomarker level of 3.4 uL of plasma on MS.

Protein	Intra-day (N=4)		Inter-day (N=20)	
	Concentration (μg/mL)	Precision (%CV)	Concentration (μg/mL)	Precision (%CV)
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

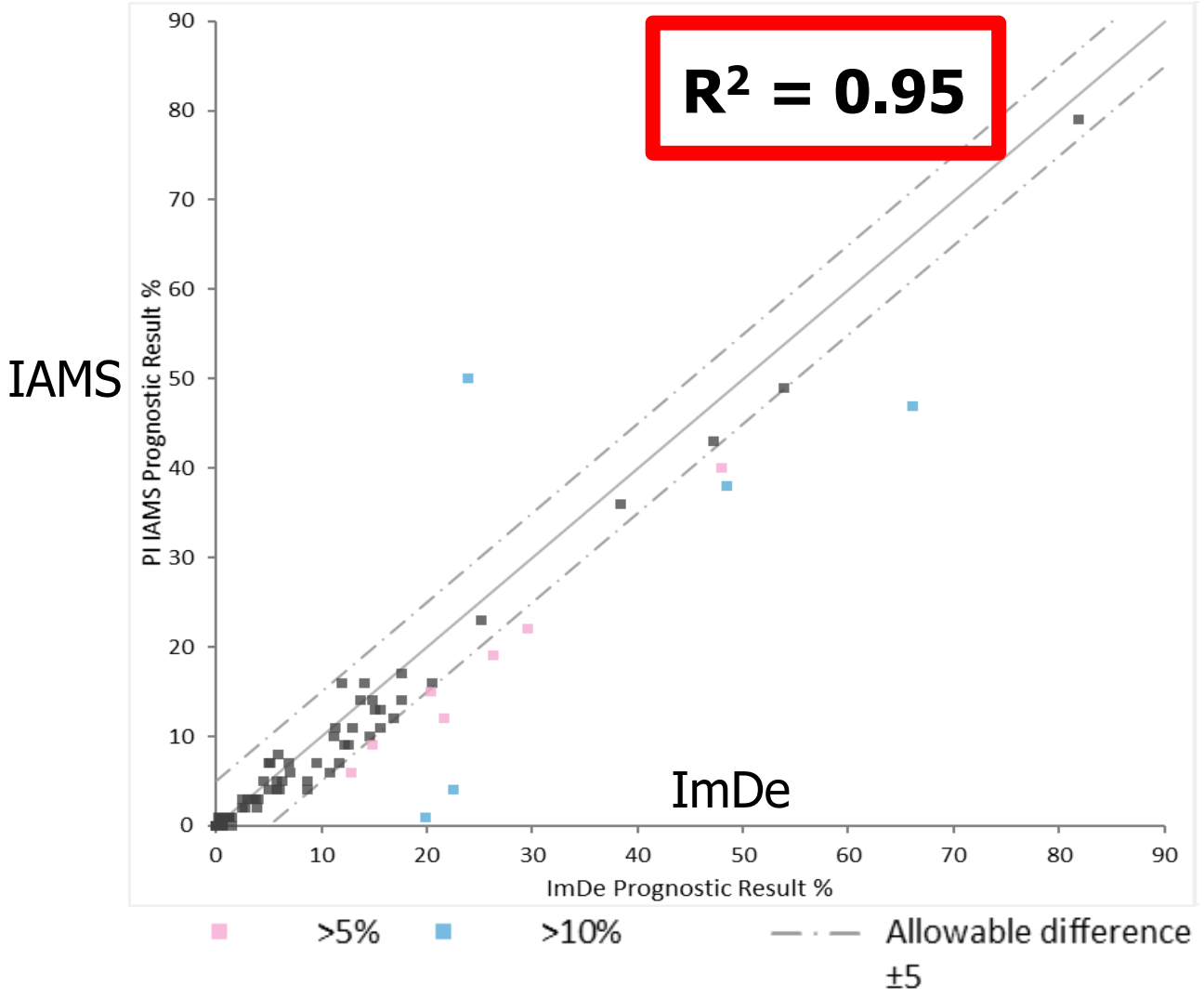
Data are mean ± SD. Precision = average percent CV. FDA acceptance criteria is <15% CV.

Cross – Platform Validation

IAMS vs ImDe

Scatter plot - high-degree of correlation between the immunodepletion method and the immunoaffinity method.

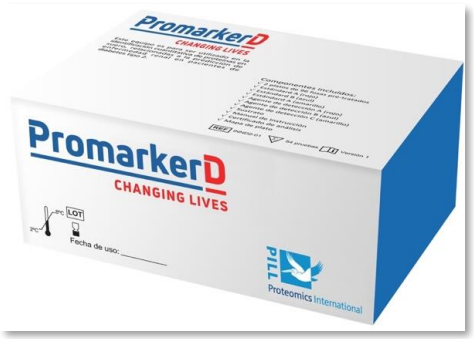
Generated after individual biomarker Bland-Altman analyses between the methods.

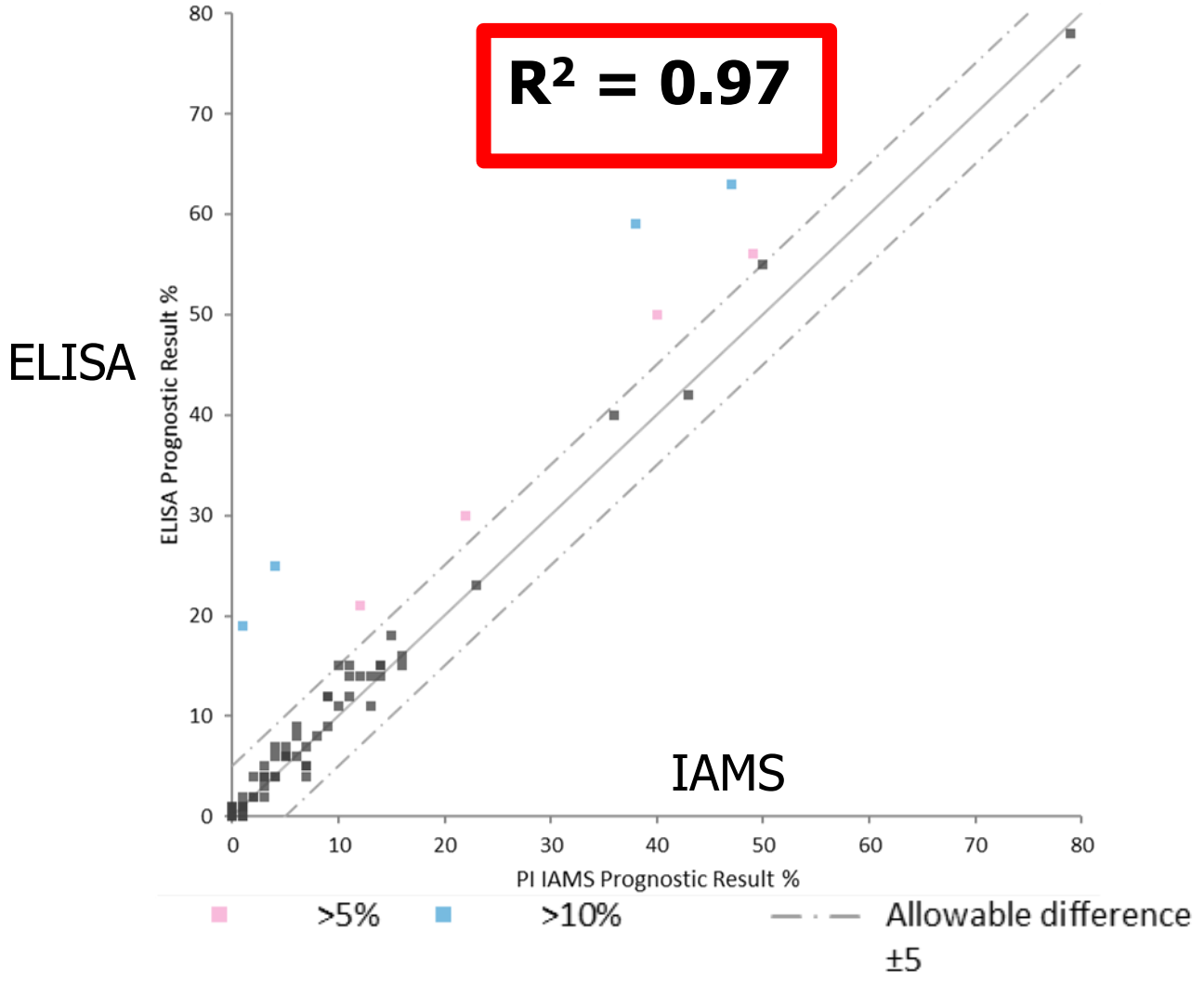


Cross – Platform Validation

IAMS vs ELISA

Scatter plot comparing PromarkerD risk predictions based on mass spectrometry and ELISA platforms.





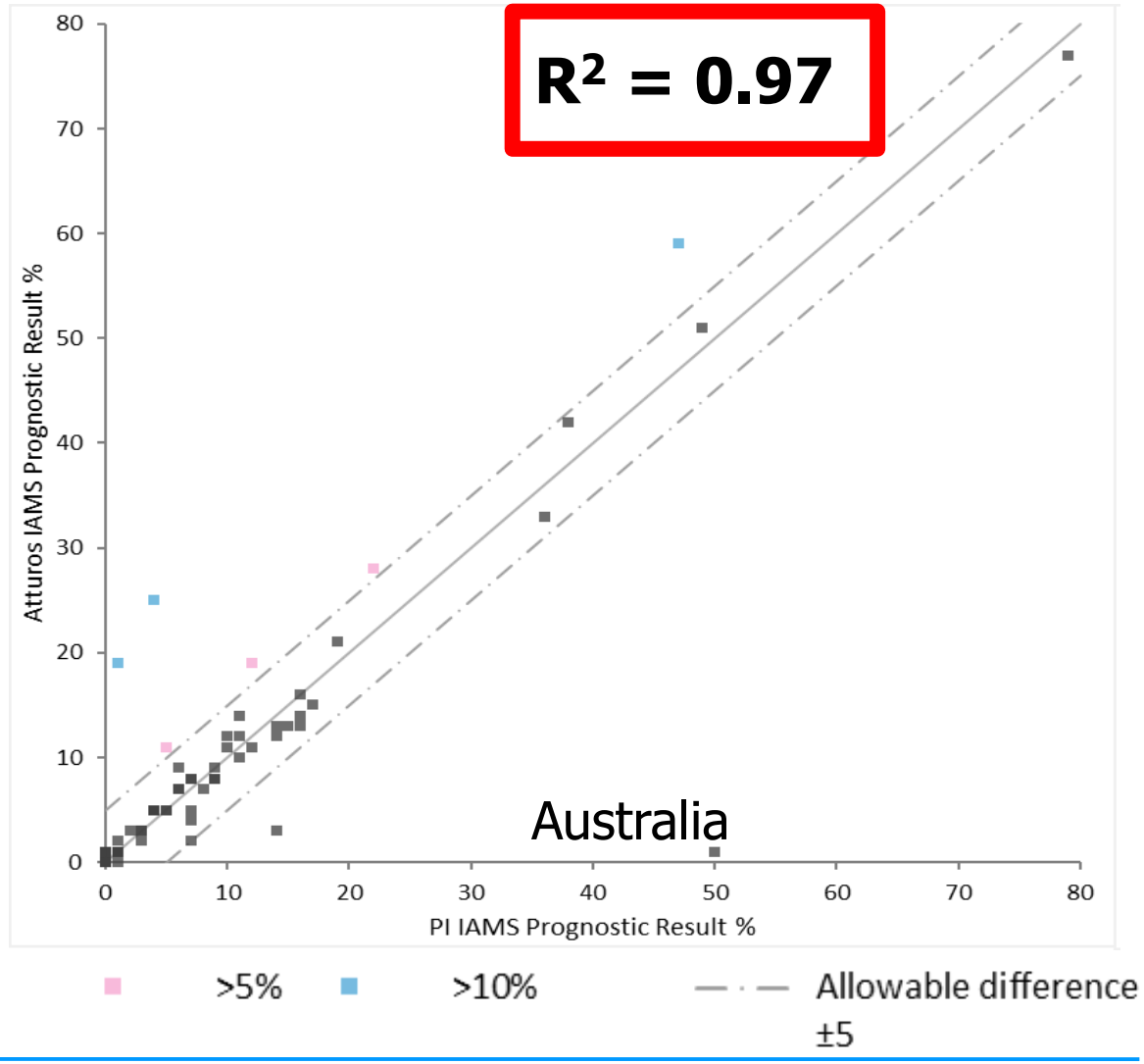
Cross – Centre Validation

Australia vs Ireland

Scatter plot - high-degree of correlation between two independent laboratories Atturos and Proteomics International with the IA-MS method

Atturos platform uses Agilent LC and Agilent 6495B MS.

Ireland



Conclusions

- Antibodies aren't just for ELISAs
- Microflow is better – sensitivity + robustness
- Immunoaffinity Mass Spectrometry can deliver
  - Sensitivity
  - Accuracy
  - Precision
  - Robustness
  - Multiplexing

PromarkerD (MS) workflow for clinical assay
as a CE mark registered prognostic test

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Thank you!

Proteomics International

Atturos MAKING BETTER DECISIONS

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