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Latest results validate biomarkers for PromarkerEndo blood test for endometriosis

- **Proteomics International's novel PromarkerEndo blood test for endometriosis advances with biomarker panel clinically validated in an independent patient group**
- **Endometriosis affects an estimated one in seven women and girls in Australia, often causing pain and infertility, with diagnosis currently taking an average of 7.5 years**
- **Latest results presented at the 29th Annual Lorne Proteomics Symposium, Victoria, Australia**

Proteomics International Laboratories Ltd (Proteomics International; ASX: PIQ), a pioneer in predictive diagnostics is pleased to announce a milestone for its novel blood test for endometriosis, PromarkerEndo, with the clinical validation of the biomarker panel in an independent patient group. The results are being presented at the 29th Annual Lorne Proteomics Symposium, the annual conference of the Australasian Proteomics Society, held 31 January - 3 February 2024.

Proteomics International's simple blood test utilises biomarkers—protein 'fingerprints' in the blood—to screen for endometriosis. The Company's prototype diagnostic test previously identified up to 90 percent of patients when comparing moderate or severe endometriosis to symptomatic controls (no endometriosis) in a study of over 901 participants [ASX: March 24, 2023].

The aim of the current study was to confirm that the panel of previously identified protein biomarkers change in concentration as severity of endometriosis increases, and this was successfully achieved. The study analysed an independent clinical cohort comprising patients obtained from the St John of God Subiaco Hospital Gynaecological Cancer Research Group [ASX: 30 June 2022], with either clinically confirmed presence (N=87) or absence of endometriosis (N=154) and healthy controls (N=47). The results demonstrated excellent statistical significance of multiple biomarkers in diagnosing endometriosis. Due to missing clinical variables and small sample size however, analysis using the previously developed prototype diagnostic model was not possible. See the attached presentation for further information.

Proteomics International's Managing Director Dr Richard Lipscombe said, *"The results presented at the conference are compelling, and further strengthen the diagnostic performance of PromarkerEndo. Confirming the clinical performance of the biomarkers in an independent patient group was a critical milestone in the development of our potential breakthrough blood test. The next step in bringing PromarkerEndo to the clinic is a larger validation study to confirm the accuracy of the test, which is already progressing. Of equal significance, we are delighted to see our Promarker™ platform performing so well and mirroring the success we see with the PromarkerEso diagnostic test for oesophageal cancer [ASX: 1 February 2024]."*

Endometriosis is a common and painful disease that affects approximately one in seven women and girls[1], often starting in teenagers (see PIQ Annual Report 2023). It occurs when tissue similar to the lining of the

uterus grows in other parts of the body where it does not belong. At the moment, there is no simple way to test for the condition, which can cause pain and infertility, and costs Australia $9.7 billion each year[1].

The current gold standard for detection is an invasive laparoscopy followed by histopathology, a surgical procedure where a camera is inserted into the pelvis through a small cut in the abdominal wall and then a biopsy is taken for analysis. On average, it takes women 7.5 years to be diagnosed[2]. PromarkerEndo could provide early screening to rule in or rule out the need for invasive surgery in women presenting with symptoms of endometriosis.

Presentation details: 29th Annual Lorne Proteomics Symposium; poster presentation [copy attached]
Title: *Validation of Biomarkers for Endometriosis*

Scott Bringans[1], Elizna Schoeman[1], Gareth Fernandez[1], Kirsten Peters[1], Kimberley Young[1], Marisa Duong[1], Martin Mead[1], Hitormi Lim[1], Richard Lipscombe[1]
[1] Proteomics International, Perth, Australia

Authorised by the Board of Proteomics International Laboratories Ltd (ASX: PIQ).

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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. Proteomics International's mission is to improve the quality of lives by the creation and application of innovative tools that enable the improved treatment of disease.

About the Promarker™ Platform
Proteomics International's diagnostics development is made possible by the Company's proprietary biomarker discovery platform called Promarker, which searches for protein 'fingerprints' in a sample. This disruptive technology can identify proteins that distinguish between people who have a disease and people who do not, using only a simple blood test. It is a powerful alternative to genetic testing. The technology is so versatile it can be used to identify fingerprints from any biological source, from wheat seeds to human serum. The Promarker platform was previously used to develop PromarkerD, a world-first predictive test for diabetic kidney disease, that is currently being commercialised. Other tests in development include for endometriosis, asthma & COPD, oesophageal cancer, diabetic retinopathy and oxidative stress.

For further information please contact:

Dr Richard Lipscombe
Managing Director
Proteomics International Laboratories Ltd
T: +61 8 9389 1992
E: enquiries@proteomicsinternational.com

Lisa Barnes
Public Relations
Profile Media
T: +61 416 583 672
E: lisab@profilemedia.com.au

Dirk van Dissel
Investor Relations & Corporate Advisor
Candour Advisory
T: +61 408 326 367
E: dirk@candouradvisory.com.au

Kyle Moss
Corporate Advisor
Euroz Hartleys
T: +61 8 9488 1400
E: kmoss@eurozhartleys.com

1 https://endometriosisaustralia.org/
2 https://www.endometriosis-uk.org/

Validation of Biomarkers for Endometriosis

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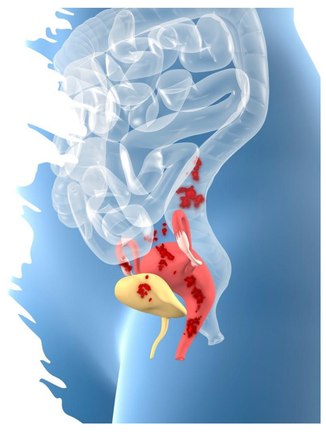
[1]Proteomics International, Perth, WA, Australia.

Proteomics International LABORATORIES LTD
www.proteomics.com.au
info@proteomics.com.au

Background

- Endometriosis, a chronic disease that is often overlooked and misdiagnosed, impacts one in ten women.
- This condition arises when cells akin to those found in the uterus start to develop in other areas of the body, typically around the pelvic region.
- The most reliable method for diagnosing endometriosis at present is through direct visualisation by histological analysis, which necessitates an invasive procedure known as laparoscopy or laparotomy.
- Globally, it's estimated that endometriosis affects 190 million women and girls.[1]
- In Australia, the economic burden of endometriosis is significant, with an annual cost of between $7.4 and $9.7 billion annually due to direct medical costs and loss of productivity.[2,3]
- **PromarkerEndo**, a targeted mass spectrometry-based assay was developed to analyse plasma samples for endometriosis.



Aim

- This study aimed to validate a panel of previously identified protein biomarkers in an independent cohort, for use in endometriosis diagnosis.

Participants and Methods

- Plasma samples (n=292) were analysed across three clinical groups:
  - **Group 1**: Endometriosis cases confirmed with laparoscopy/ histopathology (n=87),
  - **Group 2:** Symptomatic Controls with surgically confirmed absence of Endo (n=154)
  - **Group 3:** Healthy Controls (n=47) (general population, no Endo associated symptoms).

St John of God Health Care (SJOG) 2013-2016

Linear Clinical Research, Perth, Australia 2021-2022

- Four samples with unclear diagnosis were excluded from data analysis.
- In Group 2, 39% (n=60) of participants had adenomyosis.
- Average participant age was similar for Group 1 (40±7 years) and Group 2 (44±6 years), and slightly lower in Group 3 (32 ± 7 years).
- The biomarkers were originally curated from previous triplicate quantitative 2D-LCMS experiments followed by targeted mass spectrometry and correlation analysis using a large cohort (n=901).
- The targeted assay was further optimised to improve the proteomic workflow and provide greater sensitivity and reproducibility for the 47 proteins (97 peptides) in the overall assay.
- The optimised assay was run on the independent cohort (n=292).
- For statistical analysis, a natural logarithmic transformation was applied to all biomarker peak area ratios.
- Wilcoxon rank-sum tests were performed for each peptide to compare median peptide ratios across different groups. A significance threshold of $p<0.05$ was applied.

Results

- For quality control samples processed alongside clinical samples at least 67% of detectable peptides had a CV of less than 30%.
- The study identified 29 significantly different peptides median differences across 20 proteins when comparing endometriosis with healthy or symptomatic controls.
- Out of these 29 significant peptides, 18 had previously been identified as significant in a large clinical validation cohort.
- For each peptide where significant comparisons were detected, box plots marked with asterisks to denote significance were generated. As an illustration, Figure 1 shows results that provide strong evidence of a significant difference.
- Due to missing clinical variables and small sample size, external validation of previously developed multivariate models was not possible.

Results

Table 1: Peptides showing significant differences in medians in the independent SJOG cohort.

Peptide	SJOG Cohort			Clinical Validation Cohort		
	Comparison	P-value	Significance	Comparison	P-value	Significance
LnEND080Pep2	Endometriosis vs Symptomatic	0.032	*	Endometriosis vs Healthy	0.038	*
LnEND104Pep1	Endometriosis vs Healthy	0.004	**	Endometriosis vs Healthy	0.018	*
LnEND104Pep2	Endometriosis vs Healthy	0.0008	***	Endometriosis vs Healthy	0.003	**
LnEND105Pep1	Endometriosis vs Healthy	0.011	*	Endometriosis vs Healthy	0.013	*
LnEND105Pep2	Endometriosis vs Healthy	0.004	**	NS		
LnEND105Pep3	Endometriosis vs Healthy	0.011	*	NS		
LnEND120Pep1	Endometriosis vs Healthy	0.0002	***	Endometriosis vs Healthy	6.70E-08	****
				Endometriosis vs Symptomatic	0.032	*
LnEND132Pep1	Endometriosis vs Healthy	0.024	*	Endometriosis vs Healthy	3.60E-05	****
LnEND162Pep1	Endometriosis vs Symptomatic	0.017	*	Endometriosis vs Healthy	0.005	**
LnEND162Pep3	Endometriosis vs Healthy	0.04	*	NS		
LnEND170Pep1	Endometriosis vs Symptomatic	0.017	*	NS		
LnEND170Pep2	Endometriosis vs Symptomatic	0.031	*	NS		
LnEND251Pep1	Endometriosis vs Healthy	1.01E-07	****	Endometriosis vs Healthy	1.49E-09	****
LnEND251Pep2	Endometriosis vs Healthy	2.16E-07	****	Endometriosis vs Healthy	2.10E-11	****
LnEND251Pep2	Endometriosis vs Symptomatic	0.016	*	NS		
LnEND286Pep1	Endometriosis vs Symptomatic	0.002	**	NS		
LnEND318Pep2	Endometriosis vs Healthy	5.45E-14	****	Endometriosis vs Symptomatic	0.0008	***
LnEND403Pep1	Endometriosis vs Symptomatic	0.016	*	NS		
LnEND426Pep1	Endometriosis vs Symptomatic	0.04	*	NS		
LnEND440Pep3	Endometriosis vs Healthy	0.011	*	Endometriosis vs Symptomatic	0.005	**
LnEND468Pep2	Endometriosis vs Healthy	9.96E-06	****	Endometriosis vs Symptomatic	0.046	*
LnEND493Pep1	Endometriosis vs Healthy	0.04	*	Endometriosis vs Symptomatic	0.001	**
LnEND527Pep1	Endometriosis vs Healthy	3.55E-07	****	Endometriosis vs Healthy	8.28E-09	****
LnEND527Pep2	Endometriosis vs Healthy	1.52E-13	****	Endometriosis vs Healthy	3.29E-14	****
LnEND538Pep2	Endometriosis vs Healthy	0.017	*	Endometriosis vs Healthy	0.002	**
LnEND655Pep1	Endometriosis vs Healthy	3.61E-15	****	Endometriosis vs Healthy	8.63E-25	****
LnEND655Pep1	Endometriosis vs Symptomatic	0.028	*	NS		
LnEND742Pep1	Endometriosis vs Healthy	7.61E-09	****	Endometriosis vs Healthy	1.55E-55	****
LnEND746Pep1	Endometriosis vs Healthy	0.004	**	NS		

Table 1 presents the results of two separate cohorts, SJOG and a previous clinical validation cohort, where a Wilcoxon test was performed. Each row represents a specific peptide. The 'Comparison' columns indicate the groups that were compared. The 'Significance' columns denote the significance of the Wilcoxon test result, where * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, and **** represents $p < 0.0001$. NS = non-significant result.

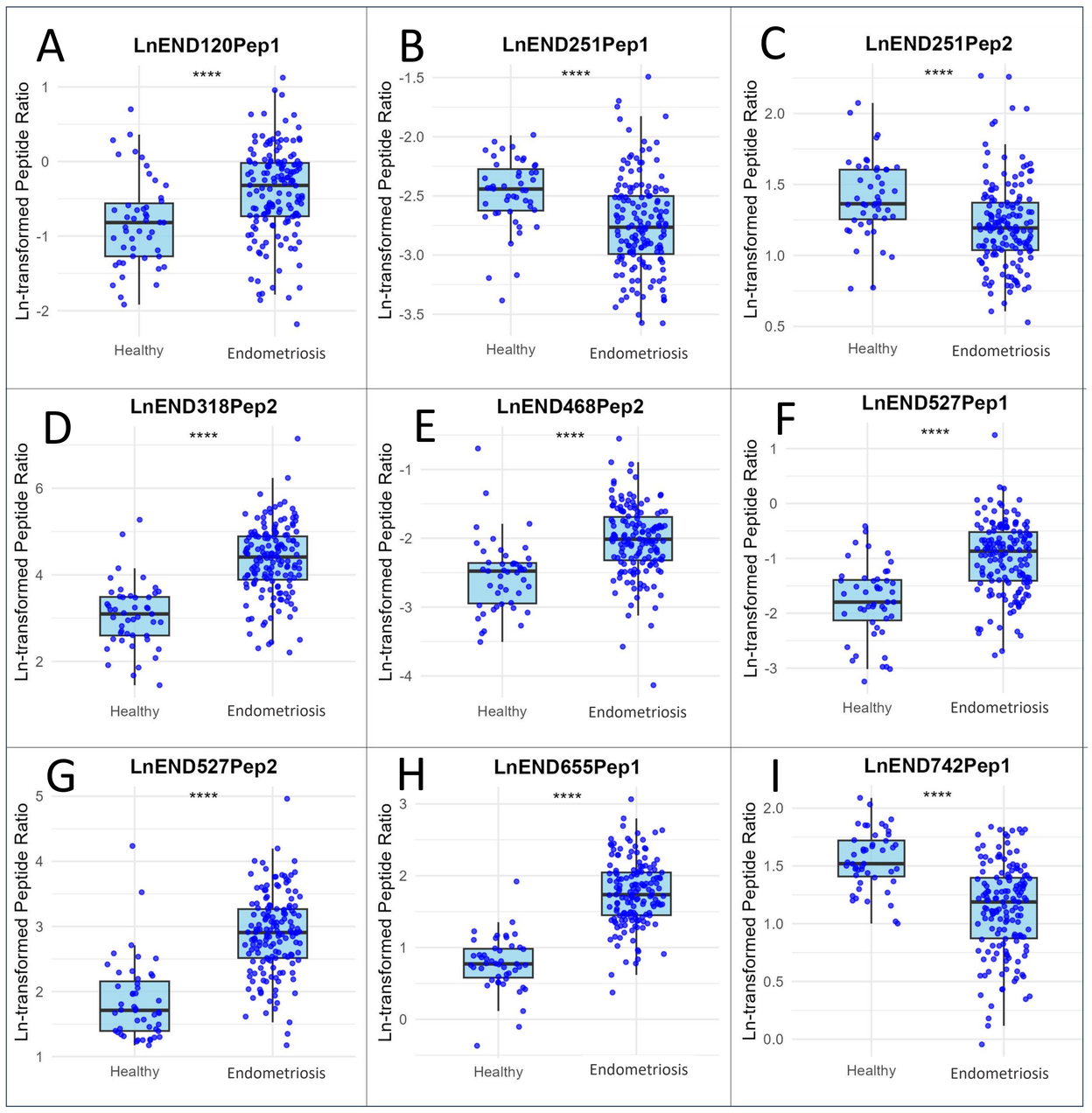


Figure 1: Box plots illustrating the distribution of Ln-transformed ratios for nine peptides with significant correlations. Panels A-I compare healthy controls to endometriosis patients for selected peptide comparisons. Each blue dot represents an individual data point. The box itself represents the interquartile range (IQR), containing the middle 50% of the data points, with the line inside the box indicating the median. Black points denote outliers. **** for p-value < 0.0001.

Conclusions

A panel of previously identified protein biomarkers, **PromarkerEndo**, were validated in this independent clinical cohort from SJOG healthcare. The results demonstrate robust evidence for inclusion of these biomarkers in a diagnostic test for endometriosis.

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[1] World Health Organisation. (2023). Endometriosis. https://www.who.int/news-room/fact-sheets/detail/endometriosis . [2] Armour *et. al.* (2019). The cost of illness and economic burden of endometriosis and chronic pelvic pain in Australia: A national online survey. PLOS ONE, 14(10), e0223316. [3] Ernst & Young. (2019). The cost of endometriosis in Australia. https://endoactive.org.au/wp-content/uploads/29May2019-FINAL-The-Cost-of-Endometriosis-in-Australia-EY-EndoActive-Report.pdf