**ASX release**
**1 August 2022**

**ASX code: PIQ**

# Potential breakthrough blood test able to detect people with endometriosis

- **Early version of potential world first non-invasive test for endometriosis correctly identified up to 78 in 100 people with the disease**
- **Diagnostic biomarkers for endometriosis were identified following a clinical validation study between Proteomics International, the Royal Women's Hospital and the University of Melbourne**
- **Results presented at the Fertility Society of Australia and New Zealand Annual Conference (FSANZ 2022), Sydney, 30 July - 2 August 2022.**
- **Endometriosis affects one in nine women and currently diagnosis typically takes an average of 7.5 years**
- **Company to take a series of steps to further refine and validate the diagnostic results to enable the commercial and clinical development of the potential breakthrough test**

Proteomics International Laboratories Ltd (Proteomics International; ASX: PIQ) is pleased to announce that an early version of the Company's potential world-first blood test for endometriosis has successfully detected up to 78 per cent of people with the painful condition. The results are being presented at the Fertility Society of Australia and New Zealand Annual Conference (FSANZ 2022) being held in Sydney, 30 July - 2 August 2022.

Endometriosis is a common and painful disease that affects one in nine women and girls, often starting in teenagers. It occurs when tissue similar to the lining of the uterus grows into other parts of the body where it does not belong. At the moment, there is no simple way to test for the condition, which often causes pain and infertility, and costs Australia $9.7 billion each year[1].

The current gold standard for detection is an invasive laparoscopy, a surgical procedure where a camera is inserted into the pelvis through a small cut in the abdominal wall. On average, it takes women 7.5 years to be diagnosed[2].

Proteomics International Managing Director Dr Richard Lipscombe said that while the test's diagnostic performance is promising, we expect it can be further developed to make it even more accurate.

"*It is exciting to have a simple blood test that may be able to correctly diagnose endometriosis in 70-80 per cent of cases. At the same time, we're optimistic we can refine the test to further improve the sensitivity and specificity, and make it more accurate for patients*."

[1] www.endometriosisaustralia.org

[2] www.endometriosis-uk.org

"*Until now, the standard of care to test for endometriosis has been through an invasive surgical procedure*," he said. *"The results are highly encouraging and a significant start in the development of a potential world first simple blood test, that could diagnose this disease earlier and without an invasive surgery".*

The test uses biomarkers—protein 'fingerprints' in the blood—to identify endometriosis. While the test is still under development, a study of the performance of an early version of the test found it could successfully distinguish between patients with and without endometriosis.

The Promarker endometriosis validation study aimed to diagnose endometriosis using a simple blood test and preliminary results showed several plasma proteins were statistically significant biomarkers for endometriosis [ASX 30 June]. The study is a collaboration between Proteomics International, the Royal Women's Hospital and the University of Melbourne [ASX: 4 August 2021].

A series of diagnostic models were developed for diagnosing endometriosis, including a comparison to symptomatic controls without endometriosis as well as differentiating disease severity. These novel diagnostic tools demonstrated that the biomarkers added statistically significant ($P<0.05$) performance to the models, with performance for sensitivity (Sn) of 65-78% across selected categories, with specificity (Sp) of 68-86%, and Area Under the Curve (AUC) scores of 0.72 - 0.89.

Dr Lipscombe said the next steps are to:

- further develop the statistical modelling to improve the tests sensitivity and specificity. This would use the 'traffic light' system developed for the Company's PromarkerD predictive test for diabetic kidney disease [PIQ Annual Report 2021];
- repeat the laboratory analysis to confirm and enhance the accuracy of the biomarker measurements. This process has already commenced, including the use of more sensitive analytical instruments;
- explore the clinical classifications of endometriosis, which is a highly complex condition, consequently diagnostics tests that selectively target sub-categories of the disease may be more accurate and provide greater clinical utility;
- to confirm the clinical performance of the test in an independent patient cohort [ASX: 30 June].

The Company anticipates that this additional analysis will be completed in stages over the next 2-6 months. If successful, the outcome would be a clinically validated blood test(s) able to offer simpler and earlier diagnosis of endometriosis or the disease's sub-categories. Proteomics International believes a validated test will garner significant interest, both commercially and in the clinic.

**Fertility Society ANZ Annual Conference 2022 poster presentation**; [copy attached; summary below]

Titled: *Biomarkers for Endometriosis*

Kerryn Garrett[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Aideen Killeen[1], Connor Laming[1], Richard Lipscombe[1], Kirsten Peters[1], Mufaidha Raju[1], Sue Wong[1], Peter Rogers[2,3], Sarah Holdsworth-Carson[2,3].

[1] Proteomics International, Perth, WA, Australia; [2]Department of Obstetrics and Gynaecology, University of Melbourne and Gynaecology Research Centre, Royal Women's Hospital, VIC, Australia; [3]Julia Argyrou Endometriosis Centre, Epworth HealthCare, Richmond, VIC, Australia

**Summary of Clinical Validation Study**

**Method**: To test the performance of the biomarkers, Proteomics International's scientists compared 872 samples across three groups: women who had been diagnosed with endometriosis through a laparoscopy (N=494), and two control groups; healthy individuals (N=153) and, importantly, patients with symptoms but no clinical diagnosis (N=242).

The clinical samples were collected over several years (2012-2017) from patients who attended a Royal Women's Hospital Pelvic Pain Clinic, and include samples from women with different grades of endometriosis and also patients with various gynaecological symptoms but no endometriosis.

**Results:** Selected diagnostic models for discriminating endometriosis and stages of disease severity

- For Healthy controls vs Endometriosis: AUC 0.89, Sn 78%, Sp 85%. Comment: it is important to note that there is a risk that because the healthy controls came from a different site that batch effects may be influencing these results.
- For Symptomatic controls (no endometriosis) vs Endometriosis positive by laparoscopy: AUC 0.72, Sn 65%, Sp 70%. Comment: an important 'real world' comparison.
- For Symptomatic controls (no endometriosis) vs Severe endometriosis positive by laparoscopy: AUC 0.84, Sn 66%, Sp 86%. Comment: indicates biomarkers are discriminating between endometriosis and non-endometriosis.

In interpreting these initial results, it is important to recognise that endometriosis is a highly complex condition with a broad spectrum of clinical indications. Consequently, endometriosis is not necessarily a simple positive versus negative test, and further work may be required to detect these subtle variations.

**Conclusions:** A series of diagnostic models were developed where novel plasma biomarkers added significant independent value to known clinical variables for diagnosing endometriosis, including comparison to symptomatic controls, and stage of severity (revised American Society of Reproductive Medicine (rASRM) guidelines). Further refinement of the diagnostic models would be beneficial to optimise rule-in/rule-out criteria.

**Glossary**

| | |
|---|---|
| Sensitivity (Sn)<br>(true positive rate) | The ability of a test to correctly identify those with the disease.<br>E.g. sensitivity of 80% means that for every 100 people with endometriosis, the test correctly diagnosed 80 with the condition. |
| Specificity (Sp)<br>(true negative rate) | The ability of the test to correctly identify those without the disease.<br>E.g. specificity of 75% means that for every 100 people with symptoms but no endometriosis, a test correctly identifies 75 as not having the condition. |
| AUC | "Area Under the ROC Curve". A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the performance of a classifier system. |
| Interpreting AUC values | Conventionally the clinical significance of AUC is:<br>> 0.7 acceptable discrimination<br>> 0.8 excellent discrimination<br>> 0.9 outstanding discrimination |

For comparison, the statistical performance of the Prostate-Specific Antigen (PSA) diagnostic test (blood test measuring the concentration of the PSA protein) for the diagnosis of prostate cancer is[3]:

- Prostate cancer versus no cancer: AUC 0.68, P = <0.001
- PSA cut-off threshold 3ng/ml: Sensitivity 32%, Specificity 87%

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

ENDS

[3] pubmed.ncbi.nlm.nih.gov/15998892/

**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 asthma & COPD, oesophageal cancer, diabetic retinopathy and oxidative stress.

**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.

**For further information please contact:**

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

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

# Biomarkers for Endometriosis

**Kerryn Garrett[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Aideen Killeen[1], Connor Laming[1], Richard Lipscombe[1], Kirsten Peters[1], Mufaidha Raju[1], Sue Wong[1], Peter Rogers[2,3], Sarah Holdsworth-Carson[2,3]**

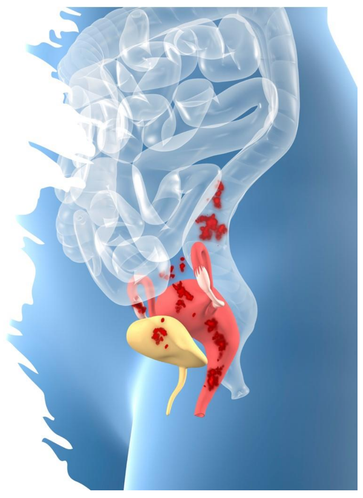
[1]Proteomics International, Perth, WA, Australia; [2]Department of Obstetrics and Gynaecology, University of Melbourne and Gynaecology Research Centre, Royal Women's Hospital, Parkville, VIC, Australia; [3]Julia Argyrou Endometriosis Centre, Epworth HealthCare, Richmond, VIC, Australia

Proteomics International LABORATORIES LTD

www.proteomics.com.au
info@proteomics.com.au

## Background

- Endometriosis is a chronic disease associated with pelvic pain and infertility, affecting one in nine Australian women today. The condition costs $9.7B/yr in direct medical expenses and lost productivity in Australia alone.
- Endometriosis is defined as the presence of endometrial-like tissue outside the uterus.
- The current gold standard for endometriosis diagnosis is visual inspection by invasive laparoscopy, preferably with histological confirmation.
- Diagnosis of endometriosis is often delayed, with an average of 7.5 years between onset of symptoms and diagnosis. There is a significant unmet need to develop a non-invasive method to diagnose the disease.



- In earlier work, a proteomics discovery platform was used to identify potential biomarkers for endometriosis. Quantitative mass spectrometry was used to analyse plasma samples from a cohort of women (n=56) across 3 clinical groups (healthy controls, no endometriosis controls, endometriosis), with 48 potential biomarkers for endometriosis identified (Figure 1).

## Aim

- This study aimed to validate plasma protein biomarkers associated with endometriosis in a large clinical cohort using a proteomics mass spectrometry workflow.

## Participants and Methods

- Plasma samples (N=901) were analysed across three clinical groups:
  - **Endometriosis** cases confirmed with laparoscopy/ pathology (N=494),
  - **Symptomatic Controls** with surgically confirmed absence of endometriosis (N=254) and
  - **Healthy Controls** (N=153).
- Endometriosis cases and symptomatic controls were recruited from patients who had endometriosis-associated symptoms (pelvic, menstrual and intercourse pain) and attended the Endometriosis and Pelvic Pain Clinic at the Royal Women's Hospital between 15 May 2012 and 17 Dec 2019. Healthy controls were recruited from the general population who had no endometriosis-associated symptoms (collected 2021-2022).
- Targeted mass spectrometry using multiple reaction monitoring (MRM) was used to validate multiple peptides for each biomarker from the Discovery phase.
- Clinical/demographic characteristics and biomarker concentrations (ln-transformed) were compared between different groups of patients in bivariate analysis using t-tests or chi-squared tests.

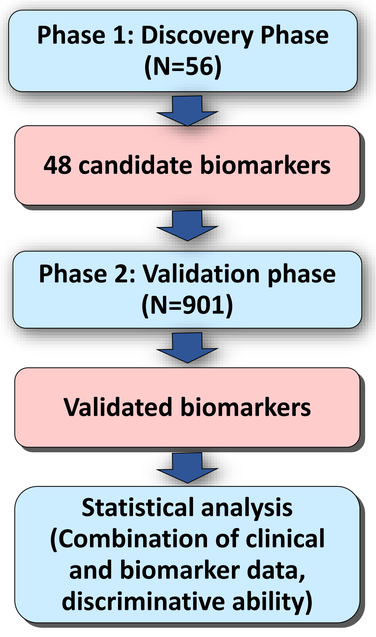



**Figure 1.** Proteomics workflow for discovery and validation of endometriosis biomarkers.

- Multivariate logistic regression was used to determine clinical associates of endometriosis, followed by inclusion of the biomarkers using a forward stepwise approach. Model performance was assessed by AUC-ROC curves (area under the receiver operating characteristic curve). The maximum Youden Index was used to determine the optimal cut-off for maximum sensitivity (Sn) and specificity (Sp) in each model.

## Sample Characteristics

- Demographic characteristics for participants in the validation phase are shown in Table 1.

**Table 1.** Demographic characteristics in the validation cohort.

| | Endometriosis (N=494) | Symptomatic controls (N=254) | Healthy Controls (N=153) |
|---|---|---|---|
| **Age** (years) | 30.3 ± 6.8 | 30.8 ± 7.9 | 28.4 ± 8.5 |
| **BMI** (kg/m$^2$) | 24.9 ± 5.2 | 27.1 ± 6.4 | 25.2 ± 5.8 |
| **Smoking status** (% Current/Ex-/Never) | 25.9/14.2/59.2 | 28.7/15.0/55.9 | 11.8/18.3/69.9 |
| **Family history of Endometriosis** (N/%) | 136 (27.5%) | 59 (23.2%) | 4 (2.6%) |
| **Age at Menarche** (years) | 12.7 ± 1.7 | 12.7 ± 1.6 | 12.8 ± 1.5 |
| **Gravidity** (sum of all pregnancies) | 0.6 ± 1.1 | 1.5 ± 2.1 | 0.6 ± 1.3 |
| **Live births** | 0.3 ± 0.7 | 0.7 ± 1.2 | 0.4 ± 0.9 |
| **Ethnicity** (% SAS/EAS/SMR/AFR/EUR/Mixed/Other) | 5.9/5.1/0.6/0.6/7 6.3/3.6/1.0 | 2.8/1.6/0.8/0.8/8 1.9/5.9/0.0 | 7.8/9.2/4.6/1.3/6 2.1/5.2/1.3 |
| **Exogenous hormone medication** (N/%) | 184 (37.3%) | 122 (48.0%) | 55 (36.0%) |
| Oral hormone medication (N/%) | 152 (20.8%) | 73 (28.7%) | 37 (24.2%) |
| Hormone IUD (N/%) | 35 (7.1%) | 44 (17.3%) | 10 (6.5%) |
| Depo injection (N/%) | 11 (2.2%) | 4 (1.6%) | 2 (1.3%) |

SAS, South Asian; EAS, East Asian; SMR, South American; AFR, African; EUR, European.
Menstrual cycle length was also considered as a clinical variable in the modelling, but due to the complexity of the data, it was reclassified into categories 0 to 4 corresponding to increasing cycle length - 0 (unknown, unsure, not cycling), 1 (14-20 days), 2 (21-27 days), 3 (28 days) and 4 (29+ days).

## Results

- From the 48 candidate biomarkers in the discovery phase, and additional literature identified markers of interest, targeted mass spectrometry assays were successfully developed for 42 proteins (represented by 78 peptides).
- Biomarkers were measured in 901 samples, with 29 samples failing QC checks, leaving 872 in the final analyses. There was no significant difference in clinical characteristics between the samples excluded and those included (data not shown).
- The disease severity of patients with endometriosis was scored and grouped by the operating surgeon using the revised American Society of Reproductive Medicine (rASRM) score. In the validation cohort, one patient was missing an rASRM score, remainder:
  - Stage I: 254 patients (51.5%) – minimal stage,
  - Stage II: 75 patients (15.2%) – mild stage,
  - Stage III: 67 patients (13.6%) – moderate stage
  - Stage IV: 97 patients (19.6%) – severe stage
- Bivariate analysis identified several statistically significant protein biomarkers (P<0.05) that could differentiate the clinical groups:
  A. Healthy Controls vs Symptomatic Controls = 18 proteins identified
  B. Healthy Controls vs Endometriosis (all rASRM stages) = 17
  C. Symptomatic Controls vs Endometriosis (all rASRM stages) = 5
  D. Symptomatic Controls vs Endometriosis Stage I+II (minimum/mild) = 11
  E. Symptomatic Controls vs Endometriosis Stage III+IV (moderate/severe) = 5
  F. Endometriosis Stage I+II (minimum/mild) vs Endometriosis Stage III+IV (moderate/severe) = 13
- A series of multivariate logistic regression models were then developed to identify:
  a) the clinical variables that could differentiate groups (A to F as described above)
  b) biomarkers which added significant independent value to the clinical variables.

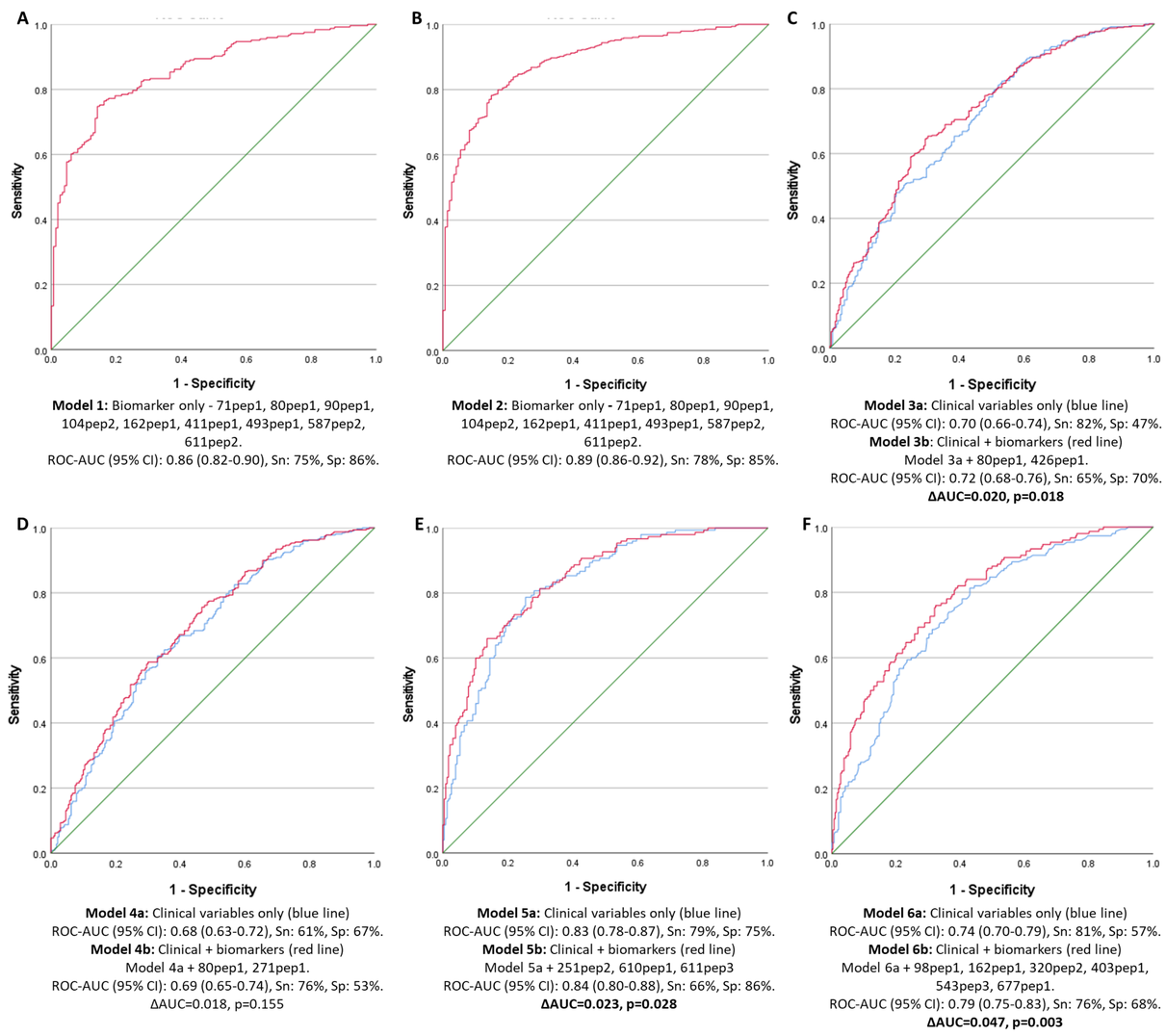



**Figure 2.** Performance of each of these multivariate models for group comparisons A to F for a) clinical and b) clinical + biomarker models for diagnosing endometriosis and differentiating the different clinical groups assessed by ROC curves. A description of each model including the protein biomarkers (peptides) included is shown below each ROC curve, together with the AUC (95% CI), sensitivity (Sn) and specificity (Sp) at the maximum Youden Index. The difference in AUC between the clinical and clinical + biomarker models is given with the associated p-value.

- For group comparisons in Figure 2A and 2B, only biomarkers were considered for entry in these models. It is important to note that there is a risk that because the healthy controls came from a different site that batch effects may be influencing these results.

## Conclusions

- A proteomics biomarker discovery workflow was used to identify and validate a panel of plasma proteins that are statistically significant biomarkers for endometriosis.
- A series of statistical models were developed where novel plasma biomarkers added significant independent value to known clinical variables for diagnosing endometriosis, including:
  - comparison to symptomatic controls
  - stage of disease severity (rASRM)
- Further refinement of the models would be beneficial to optimise rule-in/rule-out criteria.
- Endometriosis is a highly complex condition with a broad spectrum of clinical variables and further analysis is required to confirm the diagnostic application of these newly developed models.