**ASX Release**
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# New diagnostic blood test showcased at world's premier endometriosis conference

- **Proteomics International presents potential new blood test for endometriosis at the 15th World Congress on Endometriosis in Edinburgh, Scotland, held by the World Endometriosis Society**
- **Test offers improved early screening for the disease, correctly identifying up to 90 per cent of patients with the condition in a study of over 900 participants**
- **Research also suggests the current gold standard for diagnosis—an invasive surgical procedure—may be misdiagnosing some patients**
- **Endometriosis affects one in ten women often causing pain and infertility and diagnosis currently takes an average of 7.5 years**

Proteomics International Laboratories Ltd (Proteomics International; the Company; ASX: PIQ) is pleased to announce it will present its potential new blood test for endometriosis at the world's leading endometriosis conference. The world-first test will be showcased today at the 15th World Congress on Endometriosis, held in Edinburgh, Scotland, from 3-6 May 2023.

Proteomics International's simple blood test uses biomarkers—protein 'fingerprints' in the blood—to screen for endometriosis. The research to be presented at the conference indicates the strong diagnostic performance of the test, with the Company's preferred prototype correctly identifying up to 90 per cent of patients when comparing moderate or severe endometriosis to symptomatic controls (no endometriosis) in a study of over 901 participants [ASX: 24 March 2023].

Proteomics International Managing Director Dr Richard Lipscombe said we are delighted to present our potential screening test for endometriosis at the premier conference for this debilitating disease. *"To have a simple blood test, to help determine who should have an invasive laparoscopy and who should not, is an exciting development in improving the diagnosis of endometriosis. It's important these results are reviewed by the world's experts in the field as we are confident we have potentially created a new world-first blood test for diagnosing the disease."*

Endometriosis is a common and painful disease that affects one in ten women and girls, often starting in teenagers. 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 the UK economy £8.2 billion a year in treatment, loss of work and healthcare costs[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[1].

Proteomics International's test works by measuring the concentration of biomarkers in the blood that are associated with endometriosis. The Company has identified a panel of biomarkers that change

[1] www.endometriosis-uk.org/endometriosis-facts-and-figures

concentration as the severity of endometriosis increases, and analysis shows these biomarkers all relate to biological pathways that could be linked to the unwanted tissue growth that occurs in endometriosis.

Dr Lipscombe said our simple blood test can detect endometriosis, but the data also suggests the current gold standard for diagnosis—an invasive surgical procedure—may be misdiagnosing some patients, particularly in the early stages of endometriosis. The next steps in developing the test are to confirm its clinical performance and clinical utility in more people in these early stages of endometriosis or with symptoms and to look more closely at their existing diagnosis.

The World Congress on Endometriosis (WCE2023) is a key event featuring clinical and scientific progress in endometriosis and adenomyosis. The event, held every two years, is run by the World Endometriosis Society and brings together scientists, clinicians, allied health staff and patients from across the globe to advance biologic and sociologic understanding of endometriosis to improve clinical care.

Proteomics International's preferred diagnostic model targets a potential early screening test to rule in or rule out the need for invasive surgery by distinguishing symptomatic controls from moderate and severe endometriosis, and achieved sensitivity (Sn) of 90%, specificity (Sp) of 90% and an AUC (area under the ROC curve) of 0.84 [ASX: 24 March 2023].

The endometriosis diagnostic test is being developed in collaboration with the Royal Women's Hospital and the University of Melbourne [ASX: 4 August 2021].

Professor Roger Hart, Medical Director of Fertility Specialists of Western Australia and National Medical Director of City Fertility Australia, said *"Endometriosis is a chronic gynaecological condition that impacts the health of 10% of women with menstrual disturbance, pelvic pain (which is often debilitating) and infertility being the potential consequences of this condition. However, unfortunately often these symptoms are overlooked, not recognised or even downplayed by health care professionals and consequently the diagnosis and treatment is often delayed. If a simple, reliable, non-invasive test for endometriosis was developed and was readily accessible, this could be a substantial step forward to assist in the identification and subsequent management of women with endometriosis."*

**Presentation details:** 15th World Congress on Endometriosis poster presentation; [copy attached]

Title: *A Novel Plasma Protein Biomarker Test for Diagnosing Endometriosis*

Kirsten Peters[1], Elizna Schoeman[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Mufaidha Raju[1], Pearl Tan[1], Richard Lipscombe[1], Peter Rogers[2,3], Sarah Holdsworth-Carson[2,3,4].
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**Glossary**

| | |
|---|---|
| Sensitivity (Sn)<br>(true positive rate) | The ability of a test to correctly identify those with the disease.<br>E.g. sensitivity of 90% means that for every 100 people with endometriosis, the test correctly diagnosed 90 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 85% means that for every 100 people with symptoms but no endometriosis, a test correctly identifies 85 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 |

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

ENDS

**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.

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# A Novel Plasma Protein Biomarker Test for Diagnosing Endometriosis


**Kirsten Peters[1], Elizna Schoeman[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Mufaidha Raju[1], Pearl Tan[1], Richard Lipscombe[1], Peter Rogers[2], Sarah Holdsworth-Carson[2,3]**

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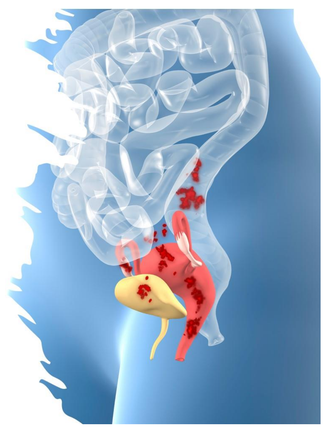
## Background

- Endometriosis is a common gynaecological condition, affecting ~10% of women globally.
- It is a major cause of female infertility and individuals can suffer from intense and chronic pain which substantially impacts quality of life.
- Delayed diagnosis (average 7.5 years), usually by invasive laparoscopy with histological verification → costly and requires a skilled physician.
- Recent guidelines from ESHRE[1] suggest imaging as an accurate alternative to laparoscopy, at least for ovarian and some cases of deep endometriosis.
- A non-invasive diagnostic test allowing earlier diagnosis is needed.



## Aim

- This study aimed to validate a panel of protein biomarkers for use in endometriosis diagnosis.

## Participants and Methods

- Plasma samples (n=901) were analysed across three clinical groups:
  - **Group 1**: Endometriosis cases confirmed with laparoscopy/ histopathology (n=494),
  - **Group 2:** Symptomatic Controls with surgically confirmed absence of Endo (n=254)
  - **Group 3:** Healthy Controls (n=153) (general population, no Endo associated symptoms).

Endo and Pelvic Pain Clinic Royal Women's Hospital, Melbourne, Australia 2012-2019

Linear Clinical Research, Perth, Australia 2021-2022

**Table 1.** Clinical and demographic characteristics for 901 participants.

| | Endometriosis (N=494) | Symptomatic Controls (N=254) | Healthy Controls (N=153) |
|---|---|---|---|
| **Age** (years) | 30 ± 7 | 31 ± 8 | 28 ± 9 |
| **BMI** (kg/m$^2$) | 25 ± 5 | 27 ± 6 | 25 ± 6 |
| **Smoking status** (% current or past) | 40 | 44 | 30 |
| **Age at Menarche** (years) | 13 ± 2 | 13 ± 2 | 13 ± 2 |
| **Family history of Endometriosis** (%) | 28 | 23 | 3 |
| **Pain** (% menstrual/pelvic/intercourse) | 93/81/76 | 93/83/77 | 0 |
| **Cycle Length** (% 14-20/21-27/28/29+/other* days) | 32/8/30/21/9 | 46/9/21/14/10 | 0/19/50/31/0 |
| **Gravidity** (% 0/1/2/3+) | 68/16/10/6 | 51/14/10/25 | 73/9/8/10 |
| **Live births** (% 0/1/2/3+) | 80/10/8/2 | 63/15/11/11 | 81/5/12/2 |
| **Ethnicity** (% AS/SMR/AFR/EUR/Other/Unknown) | 11/0.6/0.6/76/5/7 | 4/0.8/0.8/82/6/6 | 17/5/1/62/6/9 |
| **Exogenous hormone medication** (% oral/IUD/Depo inj) | 21/7/2 | 29/17/2 | 24/7/1 |

*Cycle Length other=unknown, unsure or not cycling; AS=South or East Asian, SMR=South American, AFR=African, EUR=European

- Endometriosis severity classified by the rASRM score[2]: Minimal (n=254), Mild (n=75), Moderate (n=67), Severe (n=97), and Unknown (n=1).
- A proteomics mass spectrometry platform was used to identify potential plasma protein biomarkers for endometriosis in a small discovery cohort followed by validation in a larger cohort (Figure 1).
- 51 candidate protein biomarkers were assessed.
- Clinical characteristics and biomarker concentrations were compared between endometriosis cases, symptomatic controls and healthy controls using t-tests or chi-squared tests.
- Multivariate logistic regression was used to develop models for diagnosing endometriosis 1) clinical model, 2) clinical + biomarker model.
- Model performance assessed by AUC-ROC curves (area under the receiver operating characteristic curve) and sensitivity (Sn) and specificity (Sp) determined.
- Dot plots were also used to visualise the model predictions.

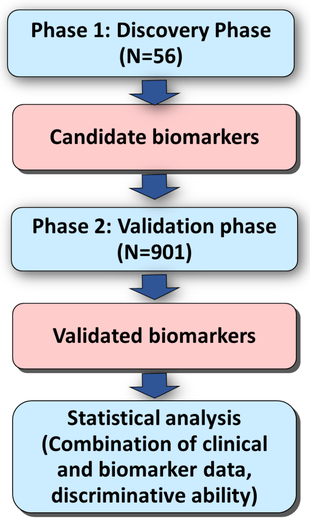



**Figure 1:** Proteomics workflow for discovery and validation of endometriosis biomarkers

## Results

- Biomarker only models to diagnose symptomatic controls or endometriosis from healthy controls had excellent AUCs (AUC=0.86 and 0.89, respectively).
- A series of clinical and clinical + biomarker models for diagnosing endometriosis were developed (Figure 2).
- After adjustment for clinical factors (age, BMI, cycle length, no. of times pregnant, hormone use and ethnicity), several biomarkers were independently associated with endometriosis and significantly improved model performance (P<0.05).


[1] Becker, et al. European Society of Human Reproduction and Embryology (ESHRE) guideline: Endometriosis. Hum. Reprod. Open **2022**, 26.
[2] Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. **1997**; 67:817-21.


## Results

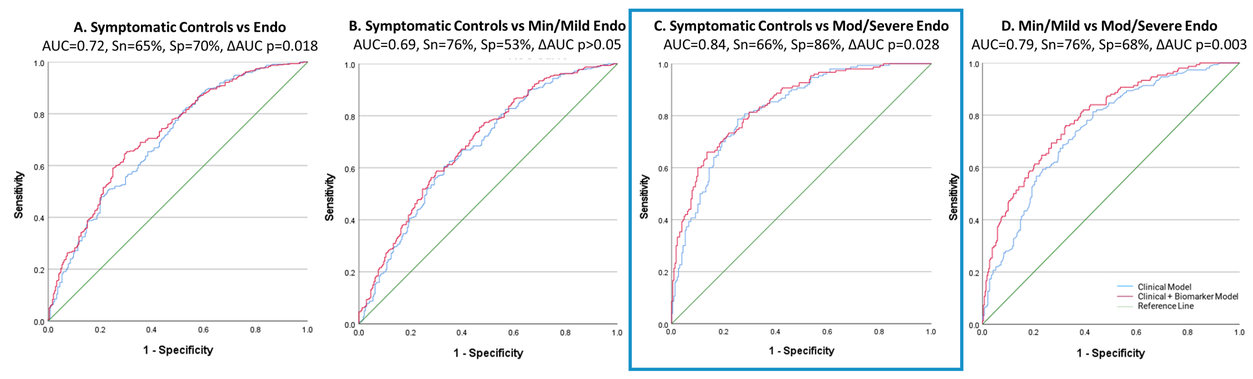



**Figure 2.** ROC-AUC curves - model performance for 1) clinical and 2) clinical+biomarker models for diagnosing endometriosis and differentiating the different rASRM stages. The AUC, sensitivity (Sn) and specificity (Sp) at the maximum Youden Index are shown, together with the p-value for difference in AUC between the clinical and clinical+biomarker models.

### Prototype → Symptomatic Controls vs Moderate/Severe Endometriosis (n=254 vs 164)

- AUC=0.84, Sn=66%, Sp=86% at max Youden Index (Fig 2C).
- Using a traffic-light approach to classify people as low, moderate or high risk using 2 cut-offs, a **prototype diagnostic test** to diagnose moderate/severe endometriosis from symptomatic controls was developed (Fig 3).
- At the optimised cut-offs, this prototype provided a Sn=90% and Sp=90%.

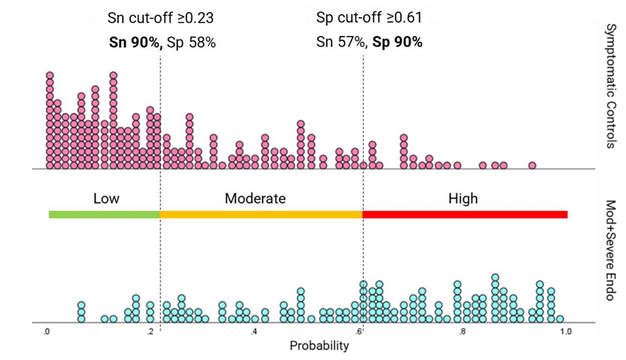



**Figure 3.** Dot plot of 'Symptomatic Controls vs Moderate/Severe Endo'. Plot shows the distribution of individuals across their predicted probabilities (prediction scores) from low to high probability for an individual to have moderate or severe Endo. The symptomatic controls in pink have lower probabilities and cluster to the left, while the moderate and severe endo cases in blue cluster more to the right with higher probabilities.

### Extremes of disease → Healthy Controls vs Severe Endometriosis (n=153 vs 97)

- AUC=0.97, Sn=89%, Sp=95% at max Youden Index (Fig 4A).
- When applying this model to the whole 901 cohort, excellent separation of healthy controls from severe endometriosis cases can be seen (Fig 4B).
- However, there are symptomatic controls with high model probabilities that map similarly to endometriosis cases across all stages from minimal to severe.
- Similarly, minimal/mild endometriosis cases with low model probabilities map like the healthy controls.
- The same overlap between symptomatic controls and the different rASRM stages was observed across other models which suggests under/misdiagnosis.

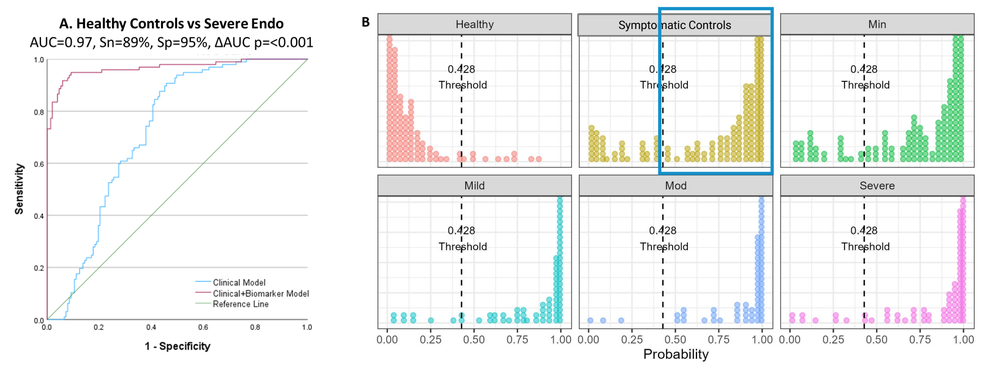



**Figure 4. A)** ROC-AUC curves - model performance for 1) clinical (age+BMI only) and 2) clinical+biomarker models for diagnosing severe endometriosis. The AUC, sensitivity (Sn) and specificity (Sp) at the maximum Youden Index are shown, together with the p-value for difference in AUC between the clinical and clinical+biomarker models. **B)** Dot plot of 'Healthy vs Severe Endometriosis' model applied to all 901 individuals. Plot shows the distribution of individuals (per clinical group) across their prediction scores from low (cluster to left) to high probability (cluster to right) for an individual to have endometriosis.

- 14 key protein biomarkers identified → coagulation and complement cascades, endoplasmic reticulum (ER) stress and unfolded protein response (UPR) signaling pathways, angiogenesis, abnormal lipid metabolism, proinflammatory, oxidative stress protection, and tissue homeostasis.

## Conclusions

- This study identified a panel of 14 plasma biomarkers that were associated with endometriosis, all with biological functions relevant to disease pathophysiology.
- A series of models were developed where biomarkers added significant value to clinical factors for diagnosing endometriosis from both symptomatic and healthy controls.
- Models have the potential to be used as a simple diagnostic blood test for endometriosis to help determine who should be referred to a specialist/have an invasive laparoscopy and who should not.
- The data also suggests that some individuals may be under/misdiagnosed in the early stages of the disease.
- Validation of these models in independent patient cohorts is required to confirm the clinical performance and clinical utility.