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World first blood test for esophageal cancer shows 94% accuracy in clinical validation study

- **Proteomics International's novel PromarkerEso blood test for esophageal adenocarcinoma shows 94% accuracy in clinical validation study**
- **Esophageal cancer is the 6th leading cause of cancer-related death, responsible for 1 in 20 cancer-related deaths worldwide, with a five year survival rate of 20% due to late diagnosis**
- **Results from 165 patient study using the world-first blood test presented at the 20th annual ISDE World Congress for Esophageal Diseases, Edinburgh, Scotland**
- **Current screening requires a specialist endoscopy procedure and the annual expenditure on treating esophageal cancer in the US is $2.9 billion**
- **Global health impact: currently 90% of esophageal adenocarcinoma cases go undetected - improved surveillance of at-risk patients using PromarkerEso could enable earlier diagnosis and significantly improve health outcomes**

Proteomics International Laboratories Ltd (Proteomics International; ASX: PIQ), a pioneer in predictive diagnostics and precision medicine is pleased to announce outstanding results for its world-first diagnostic blood test to identify esophageal adenocarcinoma, PromarkerEso. The results from the Company's clinical validation study will be presented today at the 20th annual ISDE World Congress for Esophageal Diseases, in Edinburgh, Scotland, held 22-24 September 2024.

Esophageal adenocarcinoma (EAC) is the predominant type of esophageal cancer in North America, Australia and Europe[1], and 1-2% of western population are considered at-risk for developing this cancer[2]. The prevalence of EAC has increased dramatically worldwide, with a six-fold increase over the last 40 years[3], and it has become the 6th leading cause of cancer-related death and 7th most common cancer, now affecting more than 600,000 people globally each year[4].

Proteomics International's PromarkerEso blood test utilises glycoprotein biomarkers—'fingerprints' in the blood—to screen for EAC. In this clinical validation study 165 samples (N=66 EAC; N=99 healthy controls) from the Victoria Cancer Biobank were analysed to determine which individuals had EAC and which did not. The results demonstrated accuracy of 94% and an AUC of 0.93, indicating outstanding diagnostic performance for identifying patients with EAC.

The latest study also included assessment of the refined PromarkerEso test on a previously analysed cohort (Ochsner, USA), which also resulted in strong diagnostic accuracy. These new results build on the Company's previous findings which enabled development of the prototype test [ASX: 8 September 2023] and validation

[1] N Engl J Med, 2014. **371**(26): p. 2499-509; doi: 10.1056/NEJMra1314530
[2] American Society for Gastrointestinal Endoscopy, www.asge.org
[3] Gastroenterology. 2015; 149(2): 302–317.e1; doi: 10.1053/j.gastro.2015.04.053
[4] CA Cancer J Clin 2021 May;71(3):209-249; doi: 10.3322/caac.21660

of the biomarker panel [ASX: 1 February 2024]. In total, nearly 300 samples have now been analysed across three independent patient groups.

Proteomics International Managing Director Dr Richard Lipscombe said the latest results for PromarkerEso represented an exciting breakthrough in identifying this cancer which is notoriously difficult to detect. "*We see enormous market potential for a diagnostic test to improve screening and surveillance in at-risk populations. If we can detect esophageal cancer earlier with PromarkerEso then we can help reduce the enormous cost burden of this disease on healthcare systems and save many lives.*"

Current gold-standard screening for the disease requires a specialist endoscopy, an invasive procedure that costs between £1,000-2,000 in the UK[5], and US$2,750 in the United States[6], where total expenditure on treating EAC was US$2.9 billion in 2018. In the US 1.5 million endoscopies with biopsy are performed annually in individuals with chronic acid reflux symptoms, but despite this up to 90% of EAC cases continue to go undetected[7].

The five-year survival rate for EAC is less than 20% because it is frequently diagnosed too late for effective treatment. Men over 50 with history of obesity face elevated risk of EAC, alongside risk factors such as chronic acid reflux, also known as gastroesophageal reflux disease (GERD). Barrett's Esophagus is the only known precursor to EAC, however, 95% of people with Barrett's Esophagus never develop EAC and 95% of patients diagnosed with EAC have no preceding diagnosis of Barrett's Esophagus[8].

Next Steps

PromarkerEso has patents granted in Europe, China and Australia, with other territories (including the USA) pending. Proteomics International is now preparing to launch PromarkerEso in Australia under ISO 15189 accreditation, targeting Q1 CY25, with other jurisdictions to follow in due course. In the USA, the Company will use its solid understanding of the Laboratory Developed Test (LDT) pathway and the Current Procedural Terminology (CPT) Code (reimbursement) process to accelerate commercialisation. Proteomics International is pursuing go-to-market routes comprising both traditional out-licensing and direct-to-patient strategies.

Presentation details: International Society for Diseases of the Esophagus 20th World Congress 2024, Poster 387 [copy attached; summary below]

Title: *Validation of PromarkerEso, a diagnostic blood test to identify esophageal adenocarcinoma*

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Summary of the Study

Aim: To assess the performance of the PromarkerEso diagnostic test in patients with esophageal adenocarcinoma (EAC) against healthy controls (HC).

Method: The PromarkerEso test measures the concentration of 4 glycoprotein biomarkers combined with a patient's age and BMI using a bi-model logistic regression approach. Glycoproteins are extracted from a standard blood sample using a lectin pull-down assay and their concentrations determined by mass spectrometry. The statistical model was developed by comparing N=60 EAC patients versus N=43 HC from the PROBE-NET Study, Australia (the 'development cohort').

Diagnostic performance of the model was then tested in two independent cohorts (N=10 EAC, N=14 HC [Ochsner Health System, USA] and N=66 EAC, N=99 HC [Victoria Cancer Biobank, Australia]. The PromarkerEso test results are presented using a "traffic light" system with green (low-), amber (moderate-) or red (high-risk) cut-offs to optimise test performance.

Results: In the primary clinical validation study PromarkerEso showed accuracy of 94% in both determining patients as having EAC, or not having EAC. The area under the receiver operating characteristic curve (AUC) was 0.93, sensitivity 93.1%, with low false positive rate (3.4%), and specificity 96.6% and high "rule-out" capability (NPV= 93.8%).

[5] digestivehealthyuk.com/test/endoscopy-gastroscopy/answerpack/endoscopy-faq/how-much-does-an-endoscopy-cost/

[6] www.newchoicehealth.com/endoscopy/cost, JAMA Network Open, 2021, doi:10.1001/jamanetworkopen.2021.27784

[7] RGastroenterology. 2022 Jul; 163(1): 163 ∃173; doi: 10.1053/j.gastro.2022.03.037

[8] www.cancer.org.au/assets/pdf/9-august-2020

Conclusion: This clinical validation study of PromarkerEso demonstrated excellent discrimination performance and confirms the diagnostic accuracy of this novel blood test for distinguishing patients with esophageal adenocarcinoma from healthy controls.

Glossary

Sensitivity (Sn) (true positive rate)	The ability of a test to correctly identify those with the disease. E.g. sensitivity of 80% means that for every 100 people with disease, the test correctly diagnosed 80 with the condition.
Specificity (Sp) (true negative rate)	The ability of the test to correctly identify those without the disease. E.g. specificity of 75% means that for every 100 people without disease, a test correctly identifies 75 as not having the condition.
Negative Predictive Value (NPV)	The probability that people who get a negative test result truly do not have the disease. Also known as 'rule-out' rate, it is the probability that a negative test result is accurate.
Positive Predictive Value (PPV)	The probability that a patient with a positive (abnormal) test result actually has the disease.
Probability (P)	The *P* value, or calculated *probability*, that an observation is true. Most authors refer to statistically significant as $P < 0.05$ and statistically highly significant as $P < 0.001$ (less than one in a thousand chance of being wrong).
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: > 0.7 acceptable discrimination > 0.8 excellent discrimination > 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[9]:

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

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

ENDS

About PromarkerEso

PromarkerEso is a novel blood test that measures the concentration of four glycoproteins combined with a patient's age and BMI to produce a risk score. Patients identified as high risk of having EAC are recommended for an endoscopy. Glycoprotein concentrations are measured using a proprietary mass spectrometry-based workflow. PromarkerEso test results are presented using a traffic light approach to optimise test performance with the patient ranked as low- (green), moderate- (amber), or high- (red) risk for EAC. Promarker Eso has patents granted in Europe, China and Australia, with other territories pending.

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 standard blood sample. 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

[9] pubmed.ncbi.nlm.nih.gov/15998892/; JAMA. 2005 Jul 6;294(1):66-70; doi: 10.1001/jama.294.1.66

development include for endometriosis, asthma & COPD, esophageal 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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Validation of PromarkerEso, a Diagnostic Blood Test to Identify Esophageal Adenocarcinoma

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20th ISDE World Congress for Esophageal Diseases
September 22-24, 2024 | Edinburgh, Scotland

Visit Booth #10

Background

The Problem

Esophageal Cancer

- The 6th leading cause of cancer-related death, 7th most common cancer[1].
- Affecting over 600,000 people every year globally[1].
- Poor 5-year survival rate of 20% due to late-stage diagnosis.

Esophageal Adenocarcinoma (EAC)

- Predominant type of esophageal cancer in North America, Australia and Europe[2].
- Incidence increased by 600% over the past 40 years[3].
- Barrett's Esophagus (BE) only known precursor.

Current Standard of Care

- Endoscopic screening for BE in individuals >50 years with chronic GERD symptoms is recommended[4].
  - Only 50% of EAC patients report GERD symptoms[4].
  - 90% of EAC cases continue to remain undetected[3].
  - 25% of EAC cases misdiagnosed as negative by endoscopy[5].
- Frequent routine endoscopic surveillance is invasive and costly, with misdiagnosis resulting in over-/under-treatment for EAC[6,7].
- Despite effective treatment strategies, current screening/surveillance has not impacted the rising incidence of EAC[8].

Roadblock

Solution

PromarkerEso
powered by Proteomics International

- PromarkerEso is a non-invasive diagnostic blood test for early detection of EAC.
- Early detection allows effective treatment to improve patient outcomes.

Methodology

- The PromarkerEso test was developed and validated in 292 serum samples from three independent cohorts PROBE-NET (The Progression of Barrett's Esophagus to Cancer Network), Ochsner and VCB (Victorian Cancer Biobank).
- The test differentiates two clinical groups:
  - EAC (confirmed by endoscopy-biopsy)
  - Healthy Controls (general population in PROBE-NET & VCB cohorts and negative controls by endoscopy in Ochsner cohort)

Table 1. Clinical and demographic characteristics of participants in PROBE-NET, Ochsner and VCB.

	PROBE-NET (N=103*)		Ochsner (N=24)		VCB (N=165*)	
	Development Cohort		Validation Cohort 1		Validation Cohort 2	
	EAC (N=60)	HC (N=43)	EAC (N=10)	NC (N=14)	EAC (N=66)	HC (N=99)
Age (years)	66.0 ± 11.9	57.0 ± 14.8	62.5 ± 3.0	65.5 ± 16.3	65.5 ± 11.1	35.0 ± 11.9
Sex (% male)	85	35	80	79	92	18
BMI (kg/m^2)	26.8 ± 2.7*	27.7 ± 7.1*	28.5 ± 3.8	31.1 ± 6.7	27.7 ± 4.5*	25.1 ± 4.7*

Results given in median ± median absolute deviation unless otherwise stated. EAC = esophageal adenocarcinoma, HC = healthy controls, NC = negative controls by endoscopy. * 15 participants from PROBE-NET (EAC=12, HC=3), and 18 from VCB (EAC=8, HC=10) were missing BMI and were not included in the analysis.

- The PromarkerEso test combines the concentration of 4 glycoprotein biomarkers with age and BMI using a bi-model logistic regression approach.
- Previously, the glycoprotein biomarkers were discovered using a proteomic analysis of serum samples comparing EAC and control clinical groups[9].
- In the present study, biomarkers were measured with a high-throughput mass spectrometry assay using lectin pull-down of specific glycoproteins on a magnetic bead.
- PromarkerEso test results are presented using a traffic light approach with low-, moderate- or high-risk of EAC, based on two cut-offs to optimise test performance:
  - Low/Moderate-risk cut-off – provides optimal **sensitivity** to increase true positives.
  - Moderate/High-risk cut-off – provides optimal **specificity** to reduce false positives.

Results

- Excellent discrimination performance for PromarkerEso test developed in PROBE-NET (AUC-ROC: 0.86) and validated in Ochsner (AUC-ROC: 0.82) and VCB (AUC-ROC: 0.93) (Figure 1).

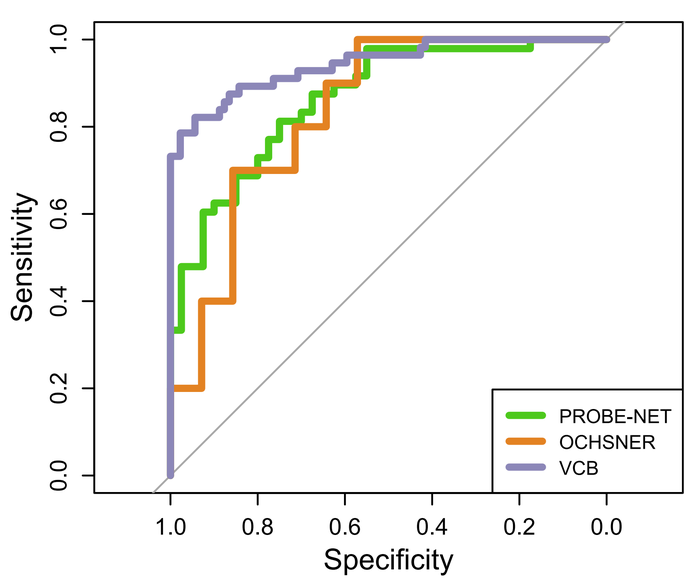

Figure 1. Performance of the PromarkerEso test using area under the receiver operating characteristic curve (AUC-ROC).

Results

PromarkerEso **Test Performance**

LOW RISK | **MODERATE RISK** | **HIGH RISK**

Cohort	Sensitivity	NPV	Specificity	PPV
PROBE-NET (Development)	100%	100%	75.0%	81.3%
Ochsner (Validation 1)	100%	100%	85.0%	80.0%
VCB (Validation 2)	**93.1%**	**93.8%**	**96.6%**	**93.9%**

Figure 2. PromarkerEso test performance. Sensitivity and negative predictive value (NPV) are assessed at the Low/Moderate-risk cut-off. Specificity and positive predictive value (PPV) are assessed at the Moderate/High-risk cut-off.

- The present validation study using samples from the Victorian Cancer Biobank shows:
  - At the Low/Moderate-risk cut-off:
    - High sensitivity, 93.1% of EAC cases classified as Moderate or High-risk.
    - High negative predictive value or "rule-out' capability, 93.8% of Low-risk results did not have EAC.
  - At the Moderate/High-risk cut-off:
    - Low false positive rate, 3.4% of healthy controls classified as High-risk.
    - High positive predictive value, 93.9% of High-risk results had EAC.

Table 2. Accuracy of PromarkerEso High-risk and Low-risk classifications by cohort.

Cohort	PromarkerEso Classification	EAC	Controls	Total	Accuracy
PROBE-NET (Development)	High-risk	**39**	10	49	**80%**
	Moderate-risk	9	16	25	
	Low-risk	0	**14**	14	**100%**
Ochsner (Validation 1)	High-risk	**8**	2	10	**80%**
	Moderate-risk	2	5	7	
	Low-risk	0	**7**	7	**100%**
VCB (Validation 2)	High-risk	**46**	3	49	**94%**
	Moderate-risk	8	26	34	
	Low-risk	4	**60**	64	**94%**

In the VCB validation cohort, the PromarkerEso test correctly identified:

- **94% of patients in the high-risk category as having EAC**
- **94% of patients in the low-risk category as not having EAC**

Conclusion

- This study confirms the diagnostic accuracy of the PromarkerEso test for EAC in two independent clinical validation cohorts.
- The PromarkerEso test is a non-invasive blood test for early diagnosis of EAC.

Acknowledgements: Analysis for this work was performed in the WA Proteomics Facility. The WA Proteomics Facility is a node of Proteomics Australia and is supported by infrastructure funding from the Western Australian State Government in partnership with Bioplatforms Australia under the Commonwealth Government National Collaborative Research Infrastructure Strategy. *References:* [1] Sung et al., (2021) CA Cancer J Clin. 71(3), 209–49. [2] Rustgi et al., (2014) N Engl J Med. 371(26), 2499-2509. [3] Rubenstein et al., (2015) Gastro., 149(2), 302-317.e1. [4] Rubenstein (2022) Am. J. Gastro. 117(11), 1759-1761. [5] Visrodia et al., (2016) Gastro., 150(3), 599-607.e7. [6] Stawinski et al., (2023) Diagnostics (Basel). 13(2), 321. [7] Shah et al., (2013) Cancer Epidemiol Bio Prev, 22 (7), 1185–1209. [8] Dam et al., (2020) Ann. Transl. Med., 8(17): 1107. [9] Shah et al., (2018) Mol Cell Proteomics, 17 (12), 2324-2334.