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A Clinical Laboratory Improvement Amendments/College of American Pathologists—Compliant Noninvasive Laboratory-Developed Test for Early Detection of Pancreatic Ductal Adenocarcinoma

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A noninvasive test for earlier detection of pancreatic cancer in individuals at higher risk is currently unavailable. We devised PancSure, a laboratory-developed test based on the protein biomarkers lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) and regenerating family member 1 β (REG1B), measured in urine by enzyme-linked immunosorbent assay, and commonly used serum/plasma carbohydrate antigen 19.9 (CA19.9), with an updated PancRISK algorithm for data interpretation. The test was validated in 565 patients: 117 asymptomatic patients without any known pancreatic condition or malignancies (21%), 242 symptomatic patients with benign pancreatic diseases (43%), and 206 patients with confirmed cancers (36%); 161 (77.5%) had stage I to II disease, and 45 (22.5%) had stage III to IV disease. PancSure passed all specifications during analytical validation and distinguishes early-stage resectable cancer from asymptomatic individuals with area under the receiver operating characteristic curve (AUC) of 0.93 (95% CI, 0.89—0.97) and 85% to 90% sensitivity (SN) and 78% to 87% specificity (SP); from symptomatic patients with AUC of 0.86 (95% CI, 0.81—0.91) and 83% to 85% SN and 72% to 83% SP; and from all noncancer patients (pooled controls) with AUC of 0.89 (95% CI, 0.84—0.93) and 83% to 85% SN and 78% to 87% SP. PancSure is a noninvasive clinical-grade test with a 48-hour turnover, ready for implementation, providing a viable solution for the earlier detection of pancreatic cancer in at-risk groups for improved patient care. *(J Mol Diagn 2025, 27: 54—61; https://doi.org/10.1016/j.jmoldx.2024.10.001)*

Despite considerable progress in our understanding of pancreatic cancer, especially its most common type, pancreatic ductal adenocarcinoma (PDAC), the disease remains lethal, with a median patients' survival of only 5 to 6 months, and exceptionally low 5-year survival of approximately 9%.[1—3] Multitude reasons for such a bleak prognosis exist—from late presentation of the disease due to lack of specific clinical symptoms in the early stages, to the currently inefficient therapeutic approaches. It is likely that both would be improved if PDAC could be detected earlier, in the presymptomatic stage. Currently, the only PDAC biomarker in widespread clinical use, serum carbohydrate antigen 19.9 (CA19.9), has false-negative results in patients with Lewis-negative genotype (5% to 10% of the White population).[4] In addition, it is elevated in various benign and malignant pancreatic and hepatobiliary diseases and unrelated cystic and inflammatory diseases, and its overall low positive predictive

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value impedes its application as a biomarker for early detection of PDAC.[5,6]

In recent years, many studies have been conducted with the aim to discover novel biomarkers to either replace or accompany CA19.9.[7–9] Among the potential candidates, the three-biomarker panel [regenerating family member 1 β (REG1B), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), and trefoil factor 1 (TFF1)] with promising characteristics has been discovered by proteomic profiling of human urine and was validated in a large cohort ($n = 371$) of control patients and patients with PDAC using an enzyme-linked immunosorbent assay (ELISA).[10] In a follow-up case-control study, the biomarker panel was tested in additional urine samples ($n = 590$).[11] In discriminating between PDAC stage I to II from healthy urine, the panel achieved area under the receiver operating characteristic curve (AUC) of 0.93 in both the training (95% CI, 0.903−0.969) and the validation (95% CI, 0.888−0.984) data sets with sensitivity (SN) and specificity (SP) > 85%. Plasma CA19.9 enhanced the performance of this panel even further, with AUC of 0.99 (95% CI, 0.983−1.00), SN of 0.96 (95% CI, 0.91−1.00), and SP of 0.97 (95% CI, 0.92−1.00). In analysis of benign versus PDAC I to II samples, the AUC for the combination of plasma CA19.9 and the panel increased from 0.9 to 0.92 (95% CI, 0.88−0.95), with SN of 0.80 (95% CI, 0.71−0.88) and SP of 0.89 (95% CI, 0.83−0.95) when compared with plasma CA19.9 and the urinary panel alone ($P < 0.001$ and $P = 0.004$, respectively).[11] Importantly, the biomarker panel combined with CA19.9 was able to detect PDAC up to 2 years before clinical diagnosis.[12] Toward easier interpretation of the data, a logistic regression-based algorithm, PancRISK, was developed, which enables stratification of patients with the binary outcome: an average or an elevated risk of developing PDAC.[13]

On the basis of these promising results and the enhanced performance of the urinary panel with CA19.9, we have now devised PancSure, a noninvasive and rapid laboratory-developed test (LDT) that uses a dedicated standardized RUO kit developed by the Diagnostic Development Hub (Singapore, Singapore). In production, the TFF1 antibody pair sourced from a different supplier than used in previous studies did not pass the necessary quality measures and produced inconsistent values in repeated assays. Therefore, TFF1 was omitted from the panel, and the RUO kit was established with ELISA assays only for LYVE1 and REG1B, to be combined with blood-based CA19.9 measured using already established assays in widespread clinical use. For test-result interpretation, PancSure uses an adapted version of the PancRISK score, which combines the levels of LYVE1, REG1B, CA19.9, and age.

Here, we report on the analytical and clinical validation of the PancSure test in a cohort of 565 subjects in a Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP)−certified environment toward clinical validity.

Materials and Methods

Clinical Samples

Urine and plasma samples used in this study were collected at Queen Mary University London (London, UK) and the Cedars-Sinai Medical Center (Los Angeles, CA) using the shared standardized operating procedures: all samples were collected before surgery or any cancer treatment and were frozen and stored at −80°C within a 4-hour window after collection. A total of 565 urine specimens (117 healthy, 242 benign, and 206 PDAC) were analyzed. Healthy cases included nine cases with familial history of PDAC. Benign (symptomatic) samples included 136 specimens collected from patients with pancreatitis, 73 with pancreatic cysts, and 33 with other benign pancreatic diseases, such as choledocholithiasis, biliary strictures, and dilated pancreatic ducts, as detailed in Supplemental Table S1. Thus, the number of high-risk cases (familial history, pancreatitis, and benign cysts) in this study totals 218 of 565 samples and constitutes approximately 39% of the cohort. Diagnosis for benign cases was performed through computed tomography as well as by pathologic examination of biopsies obtained through endoscopic ultrasound with fine-needle aspiration where appropriate. PDAC cases were represented by 161 stage I to II and 45 stage III to IV. These were histologically confirmed either from the surgical specimens or biopsy for nonresectable cases, where possible. Details of study groups, sample composition, and patient demographics are provided in Table 1. Clinical specimens were obtained after patient consent and with the approval of the respective institutional review boards: 05/Q0408/65 for Queen Mary University London and Pro00041571 for Cedars-Sinai samples.

Table 1 Demographics of Patients with PDAC and Controls

All cases		Healthy controls			Benign controls			Patients with PDAC			
Sample, *n*	Sex, *n* (%)	Sample, *n*	Age range (average), years	Sex, *n* (%)	Sample, *n*	Age range (average), years	Sex, *n* (%)	Sample, *n*	Age range (average), years	Sex, *n* (%)	Cancer stage, *n*
565	F = 305 (54) M = 260 (46)	117	23 to 83 (52)	F = 68 (58) M = 49 (42)	242	20 to 91 (60)	F = 125 (52) M = 117 (48)	206	29 to 90 (67)	F = 112 (54) M = 94 (46)	I–II = 161 III-IV = 45

F, female; M, male; PDAC, pancreatic ductal adenocarcinoma.

Immunoassays

Proprietary ELISA kits for LYVE1 and REG1B LDT were developed and manufactured by Diagnostic Development Hub and were used in all analytical and clinical performance studies. The assays measure the respective target analyte in human urine using a 96-well microplate ELISA, with separate plates for each biomarker. The REG1B assay uses an anti-human REG1B monoclonal primary antibody for target capture and a secondary monoclonal antibody pre-conjugated with horseradish peroxidase for detection (one-step sandwich ELISA). The LYVE1 assay is a two-step sandwich ELISA, which uses a monoclonal primary and secondary polyclonal antibody pair, with an additional streptavidin−horseradish peroxidase binding to biotin-labeled secondary antibody.

The absorbance was measured at 450 nm using a 96-well microplate spectrophotometer (Synergy HTX; BioTek, Winooski, VT). For both assays, the plate wells were pre-coated with the capture (primary) antibody. Commercially available Human LYVE-1 DuoSet ELISA (catalog number DY2089; R&D Systems, Minneapolis, MN) and Human REG1B ELISA Kit (catalog number SEK11638; Sino Biological, Wayne, PA) were used as third-party comparators in the analytical accuracy studies, following the manufacturer's protocols. All samples were tested in duplicates.

Plasma CA19.9 was measured by standard chemiluminescence assays using both Cobas 601E [electrochemiluminescence immunoassay (ECLIA)] platforms with the Elecsys CA19.9 kit (catalog number 11776193 122; Roche Diagnostics, Indianapolis, IN) as well as the Architect c16000 with the CA 19-9XR kit (Abbott, Des Plaines, IL).

Statistical Analysis

Quantitation of the biomarker levels was extrapolated from respective standard curves for each biomarker using a four-parameter logistic regression curve algorithm provided by MyAssays freeware (*https://www.myassays.com/four-parameter-logistic-curve.assay*, last accessed July 18, 2022). The software allows for transferring and displaying test results (analyte concentration for all samples) in an Excel sheet.

PancRISK was developed as described previously.[13] Patient age and CA19.9 values were used as continuous variables, whereas protein concentration data were natural log transformed and mean centered before the analysis. The biomarker panel was investigated for its ability to discriminate between experimental groups using a receiver operating characteristic curve analysis approach. Internal validation was performed by splitting the whole data set into the training and validation sets in a 1:1 ratio (50:50 random split at seed 111). Logistic regression was applied with a first analysis comprising healthy and PDAC samples, a second one including benign and PDAC samples, and a third analysis encompassing all control (healthy + benign) versus PDAC samples. For all three analyses, the model was fitted for the corresponding training set. Bootstrap cross-validation was used for the internal validation to ensure that overfitting was avoided. The performance characteristics of PancRISK were evaluated and compared in terms of the AUC and SNs at a fixed SPs as well as the SPs at fixed SNs being determined for all three analyses. The performance of the models designed in this study was assessed at clinically relevant SN and SP cutoffs set at >75%. The 95% CIs for AUCs were derived on the basis of DeLong's asymptotically exact method to evaluate the uncertainty of an AUC.[14] SN, SP, and 95% CI were derived using nonparametric stratified resampling with the percentile method with 100 bootstrap replicates. The method provides stable estimates with lower bias than a split-sample procedure, as described by Harrell et al[15,16] and endorsed by Steyerberg et al.[17] Positive and negative predictive values for several prevalence estimates were calculated using a standard approach.[18]

Results

LDT Analytical Validation

The developed PancSure test is based on semiquantitative ELISAs that measure the levels of two biomarkers (LYVE1 and REG1B) in urine. The analytical validation of the LDT assay included the testing of the following performance characteristics: precision, accuracy, linearity, sensitivity, specificity, reference range, and the reportable range.

Analytical Precision

The analytical precision of the developed ELISA assay was established as intra-assay and interassay CV percentages. Six different samples (two with low value, two with mid value, and two with high value for each of the three biomarkers) were evaluated. Intra-assay precision was determined by testing all six samples in three duplicate sets in a single assay run, whereas interassay precision was determined by testing the same six samples in duplicate over three consecutive assay runs. All obtained values for each biomarker are shown in Supplemental Table S2. The data are expressed as a range between the lowest and the highest CV%: for LYVE1, intra-assay CV% of 2.4% to 6.1% and interassay CV% of 2.5% to 11.3% were found, whereas for REG1B, intra-assay and interassay CV% values were 1.0% to 8.1% and 5.5% to 14.1%, respectively.

Analytical Accuracy

The analytical accuracy was determined using 60 representative urine samples: 20 from healthy donors, 20 from patients with benign pancreatic diseases, and 20 from patients with PDAC. A comparative analysis was performed

between the results generated with RUO kits and values obtained using commercially available ELISA kits. The correlation coefficients for LYVE1 and REG1B between the two sources of kits were 0.93 and 0.92, respectively (Supplemental Table S3).

Analytical Linearity

The analytical linearity of each biomarker was determined using six urine samples combined in three mixture pools (A, B, and C). Each pool was prepared using two samples, with one sample having a low biomarker value and the other one a high biomarker value (Supplemental Figure S1). Each pool was established with a highest-concentration sample, mixed with a lowest-concentration sample in five different proportions [100% (highest concentration), 75%, 50%, 25%, and 1% (lowest concentration)] according to Westgard et al.[19] The respective linearities for mixture pools (A/B/C) are as follows: LYVE1 (R^2 = 0.997/0.998/0.999) and REG1B (R^2 = 0.987/0.991/0.984).

Analytical Sensitivity

For each biomarker assay, standard curves were established with nine standard points. These were prepared by twofold serial dilution of recombinant LYVE1 and REG1B proteins (Supplemental Table S4). Using three representative lots (RUO 5, 6, and 7) in nine separate assay runs, the quantitative values of each standard's concentration were calculated and interpolated using a four-parameter logistic curve fit established for the standard curves and backfitting each standard's sample concentrations.

Applying the acceptance criteria combining the recovery percentage limits of 100% ± 20%, and assay imprecision of CV < 20.0%, the analytical accuracy for LYVE1 and REG1B standard curves [ranging from lower limit of quantitation (LLOQ) to upper limit of quantitation (ULOQ)] were 1.56 to 100 ng/mL for LYVE1 and 7.81 to 500 ng/mL for REG1B.

Analytical Specificity (Interfering Substances)

The analytical specificity of the LYVE1 and REG1B assays by interfering substances was examined, specifically hemoglobin due to hemolysis and bilirubin due to icterus that can appear in the urine of patients with PDAC. For each biomarker, three urine samples were used, representing low, mid, and high biomarker values. The sample and the interfering substances (twofold serial dilution over five points) were tested as 50%/50% mixture proportions, using stock solutions for hemolysis (hemoglobin) and icterus (bilirubin) from the Liquichek Serum Indices (catalog numbers 12012693 and 12012694, Bio-Rad, Hercules, CA). The results are shown in the Supplemental Figure S2. It is evident that no significant interference (>20%) was observed for LYVE1 and REG1B measurements with bilirubin levels up to 23 mg/dL, and for REG1B with hemoglobin levels up to 200 mg/dL. However, at high hemoglobin levels of ≥50 mg/dL, interference was observed for LYVE1 measurements: 40% at 100 mg/dL, and 93% at 200 mg/dL. The testing and interpretation of data obtained from such highly hemolytic samples would thus require special caution.

Biomarker Ranges in Urine

The reference ranges for LYVE1 and REG1B were established by testing 117 urine samples from healthy donors (Supplemental Table S5). Outliers were defined as data points that fell below quartile 1 (−1.5 interquartile range) or above quartile 3 (+1.5 interquartile range). Outliers were excluded from reference range calculation. The reference ranges for LYVE1 and REG1B were established at the 2.5th percentile distribution (lower limit) and 97.5th percentile distribution (upper limit) and were 1.56 to 79.63 ng/mL, and 7.81 to 97.93 ng/mL, respectively. Samples from healthy donors were sorted by the original sample identifier and named arbitrarily as H-001 through H-117.

The reportable ranges of LYVE1 and REG1B were fixed by the LLOQ and ULOQ determined in analytical sensitivity study and are 1.56 to 100 ng/mL for LYVE1, and 7.81 to 500 ng/mL for REG1B.

The biological ranges for the two biomarkers were set by testing 206 PDAC samples (Supplemental Table S6). Outliers were defined by data points that fell below quartile 1 (−1.5 interquartile range) or above quartile 3 (+1.5 interquartile range). The data showed non-Gaussian distribution. Thus, the biological ranges were generated at the 2.5th percentile distribution (lower limit) and 97.5th percentile distribution (upper limit) as follows: LYVE1: 3.59 to 100.00 ng/mL; and REG1B: 7.81 to 500.00 ng/mL.

LDT Clinical Validation

The LDT clinical validation was performed in a cohort of 565 patients. A significantly higher concentration of LYVE1 and REG1B was confirmed in PDAC specimens when compared with both healthy control and benign samples (Kruskal-Wallis test, $P < 0.0001$), as shown by violin plots (Supplemental Figure S3). Because of changes in the urine panel, while updating PancRISK (now based on LYVE1, REG1B, CA19.9, and age), three supervised statistical methods (logistic regression, support vector machine, and random forest) were tested and compared. The AUC values for the three statistical methods across all three comparisons [healthy versus PDAC; benign versus PDAC, and healthy + benign versus PDAC] are summarized in Table 2. As the methods performed similarly, logistic regression was used as before.[13] A summary of the performance of the adapted PancRISK in the test set (averaged across 100 random 50:50 splits) is presented as receiver operating characteristic curves (Figure 1). Supplemental Table S7

Table 2 Comparative Results of Different Supervised Statistical Methods for the Three Comparisons: H versus PDAC, Be versus PDAC, and Combined H + Be versus PDAC

Variable	H vs PDAC	Be vs PDAC	H + Be vs PDAC
Logistic regression	0.93 (0.91–0.97)	0.86 (0.82–0.92)	0.89 (0.85–0.93)
SVM	0.93 (0.91–0.97)	0.85 (0.81–0.91)	0.87 (0.83–0.93)
Random forest	0.93 (0.77–0.9)	0.86 (0.64–0.77)	0.89 (0.7–0.82)

Be, benign; H, healthy; PDAC, pancreatic ductal adenocarcinoma; SVM, support vector machine.

provides information regarding the contribution of age to the predictive model.

Table 3 shows the performance of the PancRISK for three comparisons: healthy versus PDAC, benign versus PDAC, and nonmalignant (healthy + benign) versus PDAC, for a range of fixed SNs and SPs. In healthy versus PDAC model, SNs are listed for SPs up to 99% (as opposed to the rest of the following tables) as high specificity is desired to avoid a high-false positive rate.[20]

Positive and negative predictive values for varied prevalences at fixed SN/SP across each comparison are displayed in Table 4.

Discussion

Earlier detection of PDAC will likely lead to improvement in survival of patients with this malignancy. Therefore, the quest for noninvasive biomarkers to this goal has fueled an active area of research. A noninvasive test has the clear advantage of being amenable to more frequent testing because of substantially reduced risks of procedural adverse effects, increased patient comfort, and ease of obtaining samples more rapidly and cost-effectively. Over 4000 publications on noninvasive PDAC biomarkers have been published in the past decade, with a subset of 49 articles reporting on promising biomarker candidates.[9] Of these, few are close to making their transition into a viable clinical test. This is not surprising, as the development of such a test is extremely challenging. The US Early Detection Research Network (EDRN) has described the path for biomarker development and validation as a five-phase approach: i) preclinical exploratory, ii) clinical-grade assay development and verification, iii) retrospective longitudinal trail, iv) prospective screening trial, and v) cancer control.[21] Thus, developing an LDT or US Food and Drug Administration–approvable *in vitro* diagnostic test is a time-consuming and expensive endeavor, which requires dedicated expertise and funding. Even if those prerequisites are available, many promising biomarkers fail the rigor of analytical and clinical validation steps and thus do not withstand the transition from the originating (often academic) laboratory into the clinical space. The two multicancer (including PDAC) early detection blood-based tests Cancer SEEK and Grail's Galleri and PDAC-specific Bluestar Genomics blood-based tests that have made it into clinical implementation still require detailed validation of the SN for early cancer detection.[22–24] On the other hand, the competitive performance of the IMMRay test with SN/SP of 0.84/0.98 was recently shown in a cohort comprising only approximately 35% stage I to II cases. In addition, samples with low CA19.9 values were removed from the analysis, which would be expected to increase test accuracy.[25]

The current study describes the development and characterization of a PancSure, an LDT based on ELISA

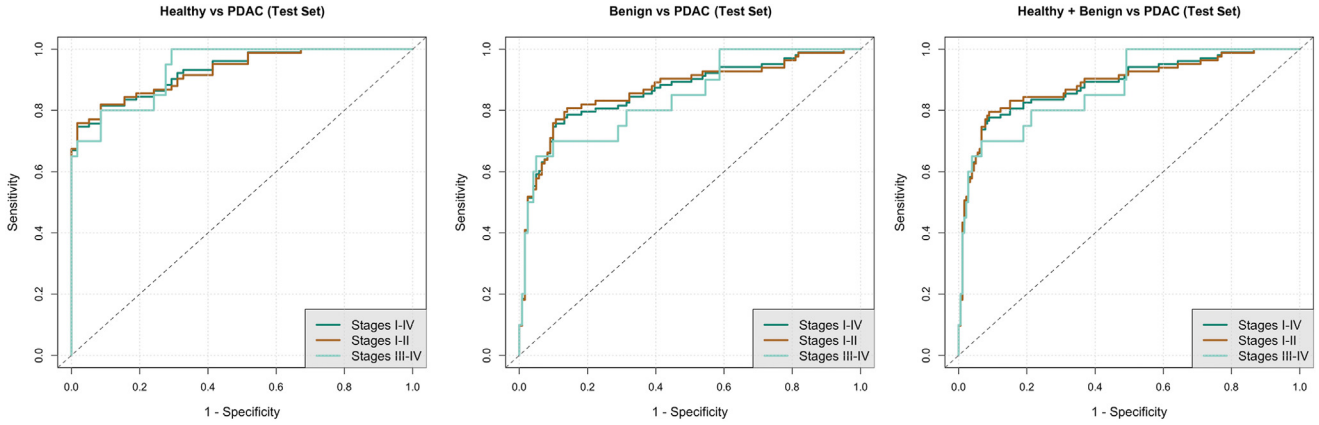

Figure 1 Performance of the PancSure test. Area under the receiver operating characteristic curve in the test (validation) set (50% of the samples) for the three diagnostic models: healthy versus pancreatic ductal adenocarcinoma (PDAC), benign versus PDAC, and pooled healthy and benign versus PDAC (for stages I to II, III to IV, and I to IV) is shown.

Table 3 Test Performance for Three Diagnostic Models: Healthy versus PDAC, Benign versus PDAC, and Healthy + Benign versus PDAC with Varied Sensitivities and Specificities

Specificity	All stages	Early stages	Late stages
Healthy vs PDAC: sensitivity based on fixed specificity			
0.75	0.92 (0.86–0.96)	0.91 (0.85–0.96)	0.96 (0.88–1)
0.80	0.89 (0.82–0.93)	0.89 (0.82–0.93)	0.93 (0.82–1)
0.85	0.86 (0.79–0.91)	0.86 (0.78–0.91)	0.87 (0.75–1)
0.90	0.82 (0.74–0.88)	0.82 (0.73–0.89)	0.81 (0.66–0.92)
0.95	0.76 (0.66–0.84)	0.77 (0.66–0.86)	0.73 (0.55–0.84)
0.96	0.76 (0.66–0.84)	0.77 (0.66–0.86)	0.73 (0.55–0.84)
0.97	0.74 (0.63–0.83)	0.75 (0.64–0.84)	0.71 (0.54–0.84)
0.98	0.74 (0.63–0.83)	0.75 (0.64–0.84)	0.71 (0.54–0.84)
0.99	0.71 (0.61–0.83)	0.72 (0.61–0.83)	0.68 (0.5–0.83)
Healthy vs PDAC: specificity based on fixed sensitivity			
0.75	0.96 (0.89–1)	0.97 (0.9–1)	0.95 (0.86–1)
0.80	0.93 (0.84–1)	0.93 (0.84–1)	0.92 (0.82–1)
0.85	0.87 (0.78–0.95)	0.87 (0.76–0.97)	0.88 (0.79–0.96)
0.90	0.8 (0.69–0.88)	0.78 (0.66–0.87)	0.83 (0.72–0.92)
0.95	0.65 (0.52–0.78)	0.6 (0.43–0.77)	0.78 (0.64–0.9)
Benign vs PDAC: sensitivity based on fixed specificity			
0.75	0.83 (0.78–0.89)	0.85 (0.8–0.9)	0.77 (0.65–0.9)
0.80	0.81 (0.75–0.87)	0.82 (0.75–0.88)	0.76 (0.65–0.86)
0.85	0.78 (0.72–0.84)	0.79 (0.72–0.86)	0.75 (0.63–0.86)
0.90	0.73 (0.65–0.82)	0.73 (0.65–0.83)	0.73 (0.6–0.86)
0.95	0.59 (0.47–0.72)	0.58 (0.45–0.71)	0.64 (0.48–0.83)
Benign vs PDAC: specificity based on fixed sensitivity			
0.75	0.87 (0.8–0.93)	0.88 (0.81–0.93)	0.84 (0.64–0.96)
0.80	0.81 (0.69–0.91)	0.83 (0.71–0.91)	0.75 (0.56–0.94)
0.85	0.69 (0.59–0.84)	0.72 (0.58–0.85)	0.65 (0.47–0.87)
0.90	0.55 (0.37–0.67)	0.54 (0.29–0.72)	0.56 (0.34–0.72)
0.95	0.35 (0.2–0.54)	0.3 (0.16–0.51)	0.47 (0.26–0.64)
Healthy + benign vs PDAC: sensitivity based on fixed specificity			
0.75	0.85 (0.8–0.9)	0.86 (0.8–0.91)	0.8 (0.68–0.91)
0.80	0.83 (0.78–0.87)	0.84 (0.78–0.9)	0.78 (0.67–0.88)
0.85	0.81 (0.75–0.86)	0.82 (0.75–0.89)	0.76 (0.62–0.88)
0.90	0.77 (0.71–0.83)	0.78 (0.7–0.84)	0.74 (0.6–0.87)
0.95	0.66 (0.57–0.75)	0.65 (0.55–0.76)	0.66 (0.52–0.79)
Healthy + benign vs PDAC: specificity based on fixed sensitivity			
0.75	0.91 (0.84–0.95)	0.91 (0.85–0.96)	0.86 (0.69–0.96)
0.80	0.85 (0.75–0.93)	0.87 (0.77–0.94)	0.78 (0.63–0.95)
0.85	0.75 (0.64–0.87)	0.78 (0.63–0.9)	0.71 (0.56–0.94)
0.90	0.62 (0.45–0.75)	0.63 (0.41–0.82)	0.62 (0.43–0.78)
0.95	0.44 (0.28–0.61)	0.39 (0.21–0.63)	0.55 (0.34–0.74)

The reported values are based on the adapted PancRISK algorithm (urinary REG1B + LYVE1 + CA19.9 + age). Early stages are stages I and II; and late stages are stages III and IV.

PDAC, pancreatic ductal adenocarcinoma.

detection of two urine biomarkers, REG1B and LYVE1. In the analytical validation of these ELISA assays, all the criteria recommended were met for precision, the interassay and intra-assay %CV are $<15\%$ and $<10\%$, respectively; the accuracy is $>90\%$; and linearity is $>95\%$ with a CV of $<15\%$ for at least three replicate measurements. As patients at various stages of PDAC may show presence of bilirubin and hemoglobin in urine, it was also crucial to determine any interference of these substances in our urine-based test. The only interference ($>20\%$) was seen with hemoglobin at high levels of ≥ 50 mg/dL when measuring LYVE1; thus, caution will have to be applied when interpreting the test results in highly hemolytic samples. With this minor exception, the devised PancSure test successfully passed all the necessary requirements and thus received approval and certification as an LDT in accordance with CLIA/CAP regulations. Phasing in the new unified ELISA kit was a necessary step in transitioning from the research-grade

Table 4 PPV and NPV for the Three Diagnostic Models for Differing Prevalences

PPV and NPV: calculated for different diagnostic models					
Model	Prevalence	Specificity	Sensitivity	PPV	NPV
H vs PDAC	0.005	0.98	0.74	0.16	0.999
	0.010			0.27	0.997
	0.020			0.43	0.995
Be vs PDAC	0.005	0.85	0.78	0.03	0.999
	0.010			0.05	0.997
	0.020			0.10	0.995
H + Be vs PDAC	0.005	0.85	0.81	0.03	0.999
	0.010			0.05	0.998
	0.020			0.10	0.995

Be, benign; H, healthy; NPV, negative predictive value; PDAC, pancreatic ductal adenocarcinoma; PPV, positive predictive value.

commercial kits to a standardized clinical assay with the objectives to secure long-term supply of raw materials and to establish quality-controlled manufacturing of the kits in sufficiently large quantities for high-volume clinical application.

For clinical interpretation of the results, an updated PancRISK score incorporates values of REG1B, LYVE1, CA19.9, as well as patients' age. Although individuals with a Lewis-negative phenotype (typically up to 10% of patients) will have no detectable CA19.9, as it is only one of the four variables, the risk score is still expected to produce the correct patient's stratification/classification. Current PancRISK differentiates early-stage malignancy from no-cancer controls with 80% to 85% SP and 82% to 84% SN (AUC, 0.89; 95% CI, 0.84−0.93).

PancSure with PancRISK algorithm is intended to be used for surveillance of high-risk groups, rather than as a screening tool in the general population, as the prevalence of PDAC is too low (approximately 12 per 100,000).[20] It is envisaged that the test will be used for stratification of asymptomatic individuals with genetic predisposition to PDAC development (familial history; gene mutations, including *BRCA1/2* and *PALB2*; and genetic syndromes, such as Peutz-Jeghers, familial atypical multiple mole melanoma syndrome, and Lynch syndrome), as well as in patients with symptoms suggestive of PDAC, and it will be based on the third predictive model that distinguished between no-cancer (healthy + benign cases) and cancer cases (including all PDAC stages). Among the symptomatic high-risk subjects, we mainly focused on patients with pancreatitis and cystic lesions, although the latter need to be examined in larger series. This will be achieved in the ongoing observational clinical study, UroPanc (*https://www.pcrf.org.uk/uropanc-study*, last accessed July 1, 2024), where PancSure and PancRISK will be externally validated in both symptomatic and asymptomatic people at risk of developing PDAC.

In conclusion, we have developed a noninvasive, clinically certified LDT with a 48-hour turnover. As ELISA is already widely used as a platform in clinical laboratories, CA19.9 is routinely measured in the clinic, and the PancRISK algorithm is based on well-proven statistical methods, PancSure can be readily implemented without the necessity of additional and costly special instrumentation. We are herewith offering a viable and long-awaited test that can likely significantly improve earlier detection of PDAC and thus care of patients with such a deadly disease.

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Disclosure Statement

T.C.-J. is a co-inventor of the relevant patent US10782301B2, licensed by Cedars-Sinai. 3rd Street Diagnostics is a business development unit for diagnostic tests within Cedars-Sinai Technology Ventures.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2024.10.001*.

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