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ORIGINAL ARTICLE

A Novel Fecal Elastase Assay for the Detection of Pancreatic Exocrine Insufficiency

Yair Huta [1], Olga Ashorov [1], Dalal Hamouda [1], Doron Boltin [1, 2], Ram Dickman [1, 2], Tsachi Tsadok Perets [1, 2]

[1] *Gastroenterology Laboratory and the Division of Gastroenterology, Rabin Medical Center, Beilinson Campus, Petach Tikva, Israel*
[2] *Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

SUMMARY

Background: **Fecal pancreatic elastase 1 (FPE1) is an established screening test for pancreatic exocrine insufficiency (PEI), a condition that is underdiagnosed and if not treated may cause significant morbidity. The aim of this study was to compare a new FPE1 machine based CLIA kit to an ELISA assay which is considered the de facto gold standard in our laboratory for FPE1 measurement.**
Methods: **Levels of FPE1 from the 227 stool samples were analyzed by the ScheBo ELISA kit and the CLIA Liaison XL system simultaneously with the same cutoff values for both assays. Performance of the Liaison XL system was assessed by calculating sensitivity, specificity, and accuracy.**
Results: **The comparison between the Liaison XL system performance and the ScheBo ELISA kit as reference revealed a sensitivity, specificity, and accuracy of 86.8%, 94.3%, and 92.1%, respectively, using a cutoff of 100 µg FPE1/g stool. When the cutoff is 200 µg FPE1/g stool the sensitivity, specificity, and accuracy were 86.6%, 97.1%, and 90.7%, respectively. Furthermore, linear correlation of FPE1 levels between the two assays were found to be significant by Pearson's correlation coefficient test (R = 0.85, p-values < 0.0001).**
Conclusions: **The Liaison XL system showed good laboratory performance with our pre-determined cutoff values when compared to our previous assay. An important advantage of this system is its semi-automated mechanism that enables large scale analysis of FPE1. In addition to that, the Liaison XL system is ideal for both qualitative and quantitative analysis of FPE1 allowing for its application to the clinical setting.**
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Correspondence:
Dr. Tsachi Tsadok Perets
Head of the Gastroenterology Laboratory
Rabin Medical Center
Beilinson Campus
39 Jabotinsky Street
4941492 Petah Tikva
Israel
Phone: + 972-3-9377297
Fax: + 972-3-9377280
Email: kaiser1974@gmail.com
tzahipe1@clalit.org.il

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INTRODUCTION

Pancreatic exocrine insufficiency (PEI) is the inability to properly digest food due to lack or inappropriate activity of the pancreatic acinar cells producing pancreatic enzymes. PEI may be caused by intra-pancreatic disorders including acute or chronic pancreatitis, autoimmune pancreatitis, pancreatic neoplasms, and Shwachman-Diamond syndrome [1-2]. Extra pancreatic disorders such as cystic fibrosis, diabetes type 1 or 2 and inflammatory bowel disease may also contribute to the

pathogenesis of PEI [3-5]. Usually, at the early stages PEI is often asymptomatic and therefore hard to diagnose. Nevertheless, without proper treatment of the underlying cause, PEI progresses and symptoms such as diarrhea, bloating, abdominal pain, and weight loss arise [6,7]. Since these symptoms are shared by many other gastrointestinal disorders, PEI patients are often misdiagnosed and left untreated. Eventually, lack of digestive enzymes including pancreatic lipase, leads to maldigestion of fat-soluble nutrients which may lead to other complications involving the hematological, cardiovascular, musculoskeletal, and immune systems [8-10]. Early detection of PEI with a suitably sensitive assay may prevent these long-term sequelae [11].

Pancreatic elastase is an enzyme that belongs to the serine proteases group which breaks down proteins such as elastin [12]. Secretion of the enzyme is controlled by several mechanisms including hormonal stimulation by cholecystokinin. In normal physiological conditions pancreatic elastase is bound to neutral steroids and bile salts and remains stable to proteolytic degradation during intestinal transit [13,14]. Furthermore, elastase is not affected by changes in transit time (increase or decrease), and therefore, measurement of its levels in the feces reflects overall pancreatic exocrine function [13-14].

The fecal pancreatic elastase 1 (FPE1) assay is an enzyme-linked immunosorbent assay (ELISA) that specifically measures pancreatic elastase 1 and is mostly used for PEI diagnosis [15]. The FPE1 enzymatic assay is non-invasive, has a high sensitivity and specificity in assessing mild to severe PEI, and it correlates well with other tests including imaging and endoscopic procedures. Another important advantage of the FPE1 enzymatic test is its simplicity which makes it cost effective and attractive when compared to alternative tests for patients with suspected pancreatic disease, such as magnetic resonance imaging or endoscopic ultrasound [16]. In addition, the assay does not require patients to change their daily routine and there are no pre-requirements such as fasting or avoidance of food of any kind prior to fecal sample collection [16,17]. Nevertheless, consideration should be applied when testing patients with diarrhea, since the quality of the assay is limited with watery fecal samples and may produce false positive results [18].

FPE1 in-vitro qualitative measurements are performed on a daily basis at the Gastroenterology Laboratory of Rabin Medical Center. The analysis of FPE1 is based on the commercially available ELISA kit Pancreatic Elastase 1™ Stool Test (ScheBo Biotech AG, Giessen, Germany) which is considered as a gold standard in many laboratories for FPE1 measurement. Although highly accurate, non-invasive and reasonably priced, this method has a few disadvantages. One of the major detriments is a low throughput per run due to limitations of the ELISA plate fixed well count. Another limitation is that the assay is performed manually which consumes both time and resources (personnel and equipment). Lastly, as with all ELISA assays, signal stability is low and a reading must be taken within minutes of adding the stop solution in order to prevent the drift of optical density data.

Chemiluminescent immunoassay (CLIA) is an alternative immunoassay to ELISA. This method is performed by a specific random-access semi-automated analyzer which increases the throughput significantly. Another advantage of the CLIA method is based on production of light from a chemical reaction which is measured by relative light units (RLU). The RLU value is proportionate to the amount of analyte present in the sample and allows for a wider dynamic range and higher concentration limit of the analyte compared to ELISA.

The aim of this study was to compare a new FPE1 CLIA kit operated by the Liaison XL analyzer (Diasorin, Saluggia, Italy) to our ScheBo ELISA assay which is considered the gold standard in our laboratory for FPE1 measurement.

MATERIALS AND METHODS

Stool sample collection

The study was conducted in our laboratory between June and August 2020 on 227 samples sent for routine FPE1 measurements. Stool samples were collected at home by all the participants using a stool collection sheet and a screw top container with a spatula. The samples deposited at a local clinic and transported to the gastroenterology laboratory using insulated, cooled thermal containers for maintaining temperature stability and FC integrity during transport. Upon arrival, samples were immediately frozen at -20°C until analyzed. All samples went through only one freeze-thaw cycle and were discarded upon completion of each test.

ScheBo ELISA FPE1 analysis

Levels of FPE1 from the 227 stool samples were analyzed by the ELISA kit Pancreatic Elastase 1™ Stool Test according the manufacturer's instruction. Briefly, Master Quick-Prep™ stool extraction devices loaded with extraction buffer were used for stool sampling. This step was followed by homogenization by a vortex and incubation for 10 minutes at room temperature (RT). Supernatants were then diluted in 1:90 ratios and 50 µL of the supernatants were loaded on the ELISA plates along four standards and two controls, and the plates were incubated at RT for 30 minutes. This step was followed by two cycles of loading reagents, incubation for 15 minutes at RT, and washing of the plates - first using POD-streptavidin and then substrate solution. Finally, stop solution was added to the plates and the optical density was measured by an iMark™ Microplate Absorbance Reader (Bio-Rad, Rishon Le Zion, Israel) at 405 nm within 5 minutes. Calculation of FPE1 in clinical samples was plotted using the standard curve created from the OD readings of the four standards.

Table 1. Liaison XL system sensitivity and specificity calculations compared with ScheBo ELISA kit results.

	Number of cases	Number of correct cases	Positive cases missed	Negative cases missed	Sensitivity	Specificity
Cutoff: 100 µg FPE1/g stool	227	209	9	9	86.8%	94.3%
Cutoff: 200 µg FPE1/g stool	227	206	18	3	84.6%	97.3%

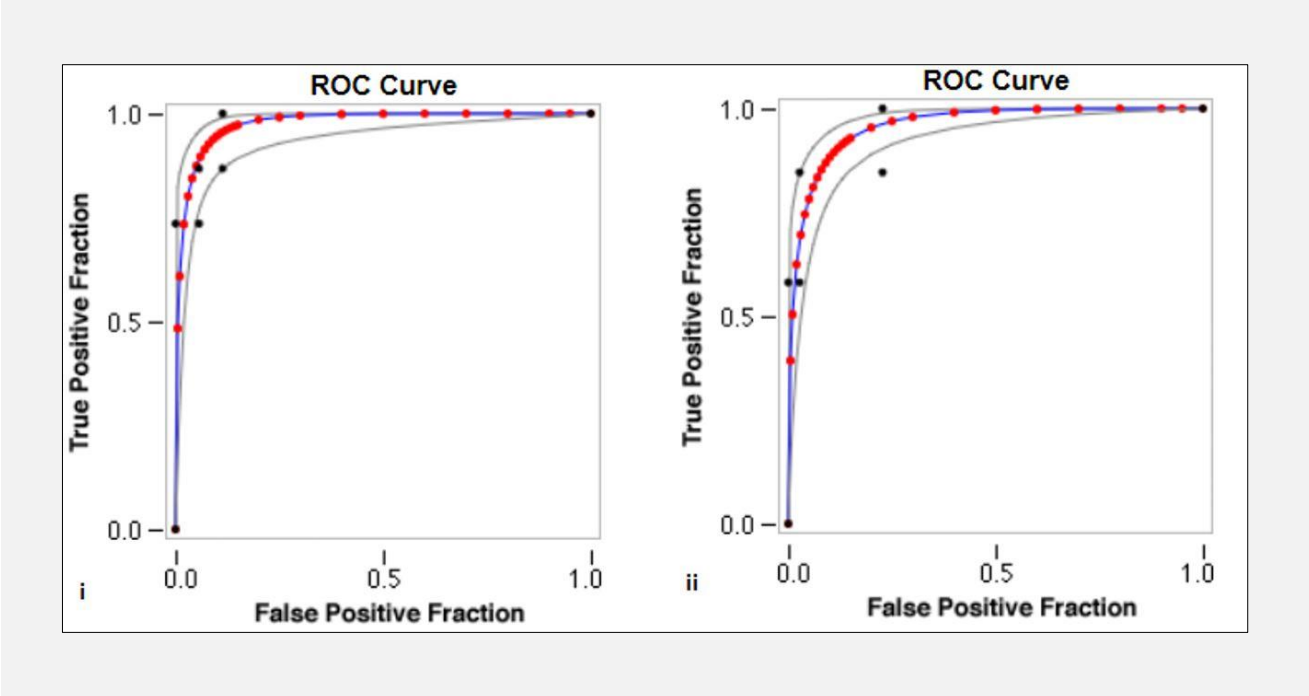

Figure 1. ROC curve analysis.

Liaison XL system versus ScheBo ELISA kit. i: Cutoff: 100 µg FPE1/g stool (accuracy = 92.1%, fitted ROC area = 0.977), ii: Cutoff: 200 µg FPE1/g stool (accuracy = 90.7%, fitted ROC area = 0.960).

Liaison XL FPE1 Analysis

The 227 stool samples analyzed for FPE1 by the ScheBo kit were evaluated simultaneously by the Liaison XL machine using the designated CLIA Liaison-Elastase1 kit in batches of 10 - 20 samples according to the manufacturer's instructions. Briefly, stool extraction devices were used for stool sampling with 6 mL of 1:5 diluted extraction buffer added to each extraction device. This step was followed by homogenization with a multi-tube vortex mixer for 30 minutes at 600 RPM. The homogenized stool extract was then loaded on suitable racks for FPE1 analysis on the Liaison XL. The same lot of the CLIA Liaison-Elastase1 kit was used for all consecutive runs.

Statistical analysis

Sensitivity, specificity, and accuracy were calculated based on the cutoff value and while referring to the ScheBo ELISA kit using a web-based calculator for ROC curves [19]. When comparing the two kits, the 227 samples were divided into four rating categories: true positives and negatives, and false positives and negatives according to the cutoff value, either 100 µg FPE1/g stool or 200 µg FPE1/g stool. Linearity between the two kits was measured using Pearson's correlation coefficient (PCC) which was calculated using a web-based calculator [20].

Ethics statement

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by

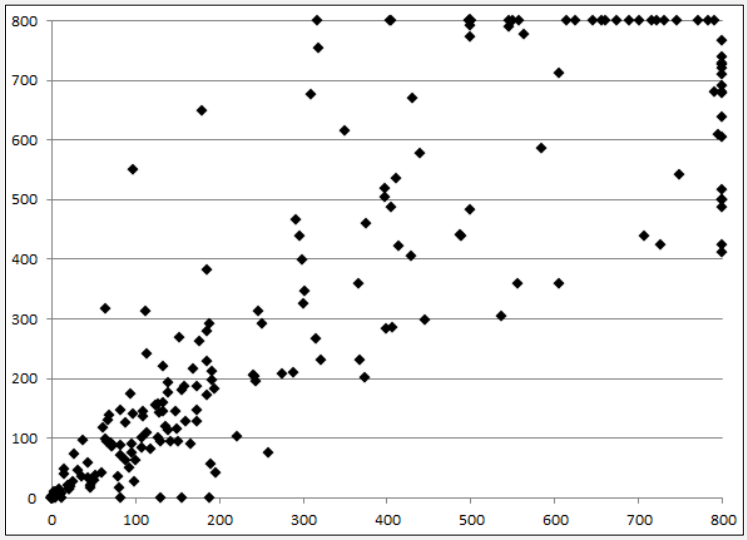

Figure 2. A graphical presentation of the linear correlation between the qualitative results of the Liaison XL system and the ScheBo ELISA kit (R = 0.85, p-values < 0.0001). X values - ScheBo ELISA kit measurements. Y values - Liaison XL system measurements.

the institutional review board of Rabin Medical Center-Beilinson Hospital (#0033-19-RMC). None of the patients in this study had actively participated in the FPE1 evaluation, nor was any data collected from them.

RESULTS

Results obtained by the ScheBo ELISA kit are classified accordingly: 200 - 500 (and above) µg FPE1/g stool - sufficient exocrine pancreatic activity, 100 - 200 µg FPE1/g stool - moderate exocrine pancreatic insufficiency, and < 100 µg FPE1/g stool - severe exocrine pancreatic insufficiency. Calculations of accuracy, sensitivity, and specificity were performed in two batches: first batch considered results as positive for PEI under 100 µg FPE1/g stool (i.e. moderate results classified as negative for PEI), and the second batch considered results as positive for PEI under 200 µg FPE1/g stool (i.e. moderate results classified as positive for PEI). Using a cutoff of 100 µg FPE1/g stool for the Liaison XL results, the sensitivity, specificity and accuracy were 86.8%, 94.3%, and 92.1%, respectively, when compared to the ScheBo ELISA kit (Table 1). Using a cutoff of 200 µg FPE1/g stool for the Liaison XL results, the sensitivity, specificity, and accuracy were 86.6%, 97.1%, and 90.7%, respectively (Table 1). Furthermore, additional analysis using a cutoff of 100 µg FPE1/g stool revealed an accuracy of 92.1% and a fitted ROC area of 0.977 and an accuracy of 90.7% and a fitted ROC area of 0.960 when a cutoff of 200 µg FPE1/g stool was applied. In order to evaluate the linear correlation between the levels of FPE1 measured by the ScheBo ELISA kit and Liaison XL, PCC was calculated and the results were confirmed as significant (R = 0.85, p-values < 0.0001) (Figure 2).

DISCUSSION

FPE1 testing is an established screening test for exocrine pancreatic dysfunction, and a useful first step for the timely detection of this under-diagnosed cause of diarrhea, bloating, and abdominal pain [21]. Causes of PEI include chronic pancreatitis, which is an established premalignant condition which requires close surveillance. Treatment of PEI is based on enzyme replacement, which not only affords patients significant symptom relief, but may help in preventing complications of PEI such as nutritional deficiencies [22]. Furthermore, FPE1 testing may serve as a treatment evaluation, evaluation of severity of disease, and follow up tool [21-22]. In this prospective study, we evaluated a new semi-automated random accessed system by comparing it to our manually handled ELISA test. Both systems are non-invasive and require no special preparation from the performing patient, such as an alternate diet or fasting, thus increase compliance of performance. During the evaluation we confirmed the efficiency and high throughput of the Liaison XL. On average, we were

able to save approximately 30 - 45 minutes in each run when compared to the ScheBo ELISA kit. In addition, the results demonstrated that the Liaison XL system had a high sensitivity, specificity, and accuracy for both values of cutoff applied - 100 or 200 µg FPE1/g stool (Table 1 and Figure 1) when compared to the ScheBo ELISA kit. Further analysis also revealed a satisfactory qualitative and quantitative correlation of 85% which was statistically significant for both cutoffs applied (Figure 2).

In summary, the Liaison XL system showed good laboratory performance with our pre-determined cutoff values when compared to our previous assay. The system is suited for measuring FPE1 levels at large scale analysis and throughput is increased significantly due to its semi-automated mechanism. The Liaison XL system is ideal for both qualitative and quantitative analysis of FPE1 allowing for its application in the clinical setting.

Declaration of Interest:

The kits for the Liaison XL analyzer were provided by Diasorin S.p.A (Diasorin, Saluggia, Italy). The authors retained full control over the design, execution, analysis and interpretation of the study, decision to publish and preparation of the manuscript. Other than stated above, the authors have no conflict of interest to disclose. All authors have approved the final version of this manuscript.

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