

General Clinical Chemistry

Evaluation of a Fecal Pancreatic Elastase-1 Enzyme-Linked Immunosorbent Assay: Assessment Versus an Established Assay and Implication in Classifying Pancreatic Function

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Running Title: Elastase-1 ELISAs and Classification of Pancreatic Function

ABSTRACT

Background: Disagreement continues regarding the two commercially available fecal pancreatic elastase-1 (PE-1) ELISAs and their respective capabilities to assess pancreatic function. Our objectives were to validate the newer PE-1 ELISA and evaluate the test against the previously established assay, to investigate the PE-1 correlation with fecal fat, and examine the PE-1 result distribution of clinical specimens.

Methods: The BioServ Diagnostics PE-1 ELISA was validated and performance characteristics compared to the previously validated ScheBo[®] Biotech PE-1 ELISA. A split sample study was accomplished using Deming regression and Bland-Altman plot analysis. Data mining was implemented to evaluate PE-1 and fecal fat correlation, and to explore PE-1 result distribution.

Results: Regression analysis shows limited quantitative agreement; slope = 0.9640, intercept = 10.787, $R^2 = 0.633$. The means were 228.8 and 226.2 $\mu\text{g PE-1/g stool}$ for the BioServ and ScheBo assays respectively. Bland-Altman analysis indicated 91% of paired values within two standard deviations of their means (100% within 2.2 standard deviations). There was good qualitative agreement between assays with 91% of cases having equivalent pancreatic function classification. The remaining 9% varied by only one classification level with no bias towards either test evident. The distribution of typical clinical specimens for infants through young adults is dichotomous, with few subjects classified with moderate pancreatic insufficiency. There is minimal agreement between PE-1 and fecal fat.

Conclusions: The BioServ Diagnostics PE-1 ELISA is an acceptable alternative to the ScheBo Biotech PE-1 ELISA. We also recommend that PE-1 replace fecal fat analysis for the evaluation of pancreatic function.

Nonstandard abbreviations: PE-1, pancreatic elastase-1; FDA, US Food and Drug Administration; AMR, analytical measurement range.

The evaluation of exocrine pancreatic function previously required invasive diagnostic approaches such as the secretin-pancreozymin or Lundh tests, or indirect methods such as the fecal chymotrypsin, bentiromide, pancreolauryl tests and timed fecal collections for fat analysis (1). With the detection of human pancreatic elastase-1 (PE-1) in both human pancreatic secretions and feces (2), and the discovery that PE-1 concentrations in feces are five to six times greater than those in pancreatic juice (3,4), it was proposed that the PE-1 concentration in feces mirrors pancreatic function (5).

With the finding that PE-1 does not degrade during intestinal transit together with the development of a stool test for measuring the enzyme, several studies have evaluated the diagnostic efficiency of fecal PE-1. These studies report both the sensitivity and specificity to be greater than 90% for the diagnosis of pancreatic insufficiency (5-11). As a result, the PE-1 stool test is used for the diagnosis of, or exclusion of exocrine pancreatic insufficiency caused by chronic pancreatitis, cholelithiasis, cystic fibrosis, diabetes mellitus, papillary stenosis and pancreatic cancer (5-22). Studies also report fecal PE-1 to be equivalent or superior to fecal chymotrypsin, lipase and pancreolauryl tests (8,9,20,21).

Currently, there are two PE-1 stool ELISAs commercially available: The BioServ Diagnostics Fecal Elastase-1 ELISA stool test (BioServ Analytics and Medical Devices Ltd., Rostock, Germany) marketed in the USA through Joli Medical Products Inc. (Willamsville, NY), and the ScheBo[®] Pancreatic Elastase-1 Stool Test (ScheBo[®] Biotech AG, Giessen, Germany) provided in the USA by ScheBo Biotech USA Inc. (Marietta, GA) but exclusively available from Genova Diagnostics[™] (Asheville, NC). Both manufacturers are registered with, and their test kits listed with the US Food and Drug Administration (FDA).

The tests involve PE-1 extraction from a small portion of stool into a buffer solution followed by dilution and then analysis by ELISA (23,24). Both ELISAs are based on the “sandwich” immunoassay technique, with the ScheBo ELISA utilizing monoclonal antibodies (24) and the BioServ ELISA polyclonal antibodies (23). The antibodies are specifically directed, recognizing defined peptide sequences of the human PE-1 molecule. Because elastase enzyme supplements are of animal origin

and the assays are specific for human PE-1, patients receiving enzyme substitution therapy do not need to interrupt their supplementation when tested (5,10,11,23,24).

Both tests are designed to have an analytical measurement range up to 500 µg PE-1/g stool, and have reference intervals of <100, 100 – 200, and >200 µg PE-1/g stool for severe pancreatic insufficiency, mild or moderate insufficiency, and normal pancreatic function respectively (23,24). Some studies suggest that the BioServ assay may exhibit a slightly greater diagnostic sensitivity compared to the established ScheBo assay (68.9 and 77.8% respectively) while maintaining essentially an equal specificity of 76 to 77% (25, 26).

Since the signing of an exclusive licensing agreement between ScheBo Biotech and a USA based diagnostic laboratory, there has been some dispute as to the equivalency between these two non-invasive PE-1 assays. Since we have validated and employed both the ScheBo and BioServ tests in our laboratory, we have taken the opportunity to address the issue.

MATERIALS AND METHODS

BioServ and ScheBo PE-1 ELISA kits were purchased from the US distributors cited previously. SPECTRAMax[®] PLUS plate readers were manufactured by Molecular Devices Corp. (Sunnyvale, CA) and PC controlled using Molecular Devices Corp. ProMax software. Data analysis was performed using Microsoft Corp. Excel software (Bellevue, WA).

Stool specimens sent to ARUP Laboratories for PE-1 analysis were collected and stored short term (< 1 week) at 4 °C or frozen at -20 °C for longer storage. Specimens were deidentified using Internal Review Board approved protocols, IRB #7275.

PE-1 extractions were performed by the ARUP Microbial Antigen Detection Laboratory following instructions provided by each kit manufacturer. ELISA analyses were performed by the ARUP Institute for Clinical and Experimental Pathology or the ARUP Special Chemistry Laboratory as

instructed by the kit manufacturers (23,24). Unless otherwise noted, samples were run in duplicate and results reported as the mean of the two measurements.

PE-1 ELISA kit comparison studies were accomplished using Deming Regression and Bland-Altman Plot analysis. Because our emphasis was to scrutinize these assays in classifying pancreatic insufficiency at and near the critical cut-off values (<100, 100 - 200 and >200 µg PE-1/g stool; severe, moderate and normal respectively), results exceeding the analytical measurement range (AMR) of 500 µg PE-1/g stool were excluded in the comparison studies.

Patient PE-1 and fecal fat testing results were extracted from the ARUP database. The data were deidentified under the Internal Review Board approval stated previously. For assessing the relationship between PE-1 and fecal fat, results were limited to those subjects having had the two tests within a three month time period. Fecal fat measurements were converted to percentages. In addition, the distribution of all PE-1 results for patients 25 years or younger obtained by our laboratory from July 2003 through October 2004 was examined.

RESULTS

BioServ PE-1 ELISA validation studies generated the following assay performance characteristics. An analytical sensitivity or detection limit of 4 µg PE-1/g stool was calculated (mean plus two standard deviations) from the measurement of ten replicates of the zero calibrator. (The kit manufacturer claims 5.5 µg/g.) This results in an AMR of 4 to 500 µg PE-1/g stool. Our validation showed the BioServ assay to not be linear throughout the entire AMR. However, in the critical range of 4 to 200 µg PE-1/g stool, linearity was acceptable generating a slope and intercept of 0.99 and 15.6 respectively ($R^2 = 0.97$, $n = 6$).

Assay imprecision was examined at three levels. Within-run precision was calculated at 115 ± 4.3 , 204 ± 6.8 and 361 ± 10.8 µg PE-1/g stool with CVs < 4%. Between-run precision was found to be 118 ± 15.6 , 176 ± 16.6 and 398 ± 33.8 µg PE-1/g stool generating CVs from 8 to 13%. A comparison of the

performance characteristics of the BioServ assay with those derived from our earlier validation of the ScheBo assay is summarized in Table 1.

Of 68 specimens assayed using both the BioServ and ScheBo ELISAs, 35 fell beyond the shared 500 $\mu\text{g/g}$ upper AMR of the assays. These results were excluded from Deming Regression and Bland-Altman analysis.

Deming Regression of the remaining 33 results produced a slope of 0.96 and intercept of 10.8 ($R^2 = 0.63$) as illustrated in Figure 1. Mean values of 228.8 and 226.2 $\mu\text{g PE-1/g stool}$ resulted for the BioServ and ScheBo assays respectively. The standard error of the estimate was 105.8 $\mu\text{g PE-1/g stool}$.

The Bland-Altman Plot analysis in Figure 2 shows 91% of the paired PE-1 values to be within two standard deviations of the difference from their means. Increasing the limit to 2.2 standard deviations included 100% of the values.

In assessing qualitative pancreatic function, there was a 91% agreement (62 of 68 paired results) between the two assays. The remaining 9% (six paired results), differed by one classification level leaving none varying by two levels, i.e. normal function and severe pancreatic insufficiency. In addition, neither test was predominantly biased over the other in the six inconsistencies, with each ELISA categorizing three subjects with a higher level of pancreatic sufficiency over the other (Table 2).

The cumulative frequency plot shown in Figure 3 illustrates the PE-1 distribution for patients 25 years of age or younger measured at our facility from July 2003 through October 2004 ($n = 400$). Results $< 15 \mu\text{g PE-1/g stool}$ (ScheBo ELISA limit of detection as listed in reference 24) are shown as 15 $\mu\text{g/g}$. Those $> 500 \mu\text{g/g}$ are plotted at the 500 $\mu\text{g/g}$ concentration. The graph demonstrates the distribution to be dichotomous, with 15% of patients having PE-1 concentrations $< 100 \mu\text{g/g}$, 78% $> 200 \mu\text{g/g}$ and 7% between 100 and 200 $\mu\text{g PE-1/g stool}$.

The graph in Figure 4 plots the measured PE-1 versus the percent fecal fat for 44 subjects having had the two tests performed within three months of each other. With the exception of two cases

suggesting that very high fecal fat may correlate with low PE-1 concentration, no relationship appears to be evident.

DISCUSSION

During recent years, the utility of fecal PE-1 in assessing pancreatic function has been well established (5-22,25,26). The noninvasive nature of fecal PE-1 testing and ease of specimen collection, in addition to its equivalence and/or superiority versus other pancreatic functional tests, demonstrates PE-1 to be of significant benefit for patients suffering pancreatic abnormalities (8,9,20,21). For example, a recent report demonstrated the value of PE-1 for correctly categorizing pancreatic functional status in cystic fibrosis patients previously misclassified using alternate methods (22). In other words, the advantages of fecal PE-1 are well supported at this time. However, there is some dispute as to the equivalency between the two ELISA kits available for quantifying fecal PE-1.

Given that our laboratory has validated and utilized both the ScheBo and BioServ PE-1 ELISAs for patient care, we are able to address issues as to how the two tests compare. In addition, there have been some misconceptions and contradictions circulating associated with the FDA status of these two PE-1 assays, which we have researched and would first like to clarify.

To begin with, Section 510 of the Federal Food, Drug and Cosmetic Act requires device manufacturers and distributors to register their establishments and list their devices they have in US commerce with the FDA (27). As stated previously, the manufacturers and/or distributors of the ScheBo and BioServ PE-1 ELISAs have complied with this requirement (September 14, 2000 and March 4, 2003 respectively). In addition, both tests have been declared by the FDA to be exempt from 510(k) premarket notification. Under the FDA's Modernization Act of 1997, 510(k) exempted products continue to be regulated by the FDA and remain subject to good manufacturing practice regulations, FDA factory inspections and other general controls. Moreover, an FDA premarket exemption allows manufacturers to market exempted products without clearance from the FDA (28).

[ScheBo submitted a premarket notification March 12, 2001, K#: K010736. The test was classified at that time as Class II, classification regulation number 864.6550. Upon review however, the FDA determined the test exempt from premarket notification requirements and reclassified the device as Class I, classification regulation number 862.175. Consequently, 510(k) clearance was not granted because it was no longer required for marketing the device. The FDA notified ScheBo of the decision by letter dated May 11, 2001. A copy of this letter was obtained under the Freedom of Information Act.]

Up until a few months before submission of this manuscript, the laboratory exclusively offering the ScheBo PE-1 test in the US (Genova Diagnostics) was declaring the ScheBo PE-1 stool test as the “Only FDA-approved elastase test for pancreatic exocrine insufficiency”. Furthermore, BioServ was also claiming their PE-1 assay as “FDA approved”. However, FDA regulatory requirements state that establishment registration and medical device listing does not constitute marketing clearance by the FDA (27). In addition, FDA regulations state that “FDA approval” is reserved for Class III devices (27), a class to which neither of these assays belong to since both are classified by the FDA as Class I devices [classification regulation number 862.1725 (29)]. Therefore, with respect to these two PE-1 ELISAs and their status with the FDA, it is concluded that statements such as “FDA cleared” or “the only FDA approved test” are misleading as supported by the information presented above. It is therefore appropriate that the majority of these declarations have recently been rectified. [ScheBo continues to proclaim their test as “FDA cleared” (30).]

In summary, both the ScheBo and BioServ PE-1 assays are listed as required with the FDA, and are allowed by the FDA to be legally marketed in the US without FDA premarket clearance or FDA approval because of their 510(k) exempt status. In other words, both tests are equivalent with respect to their standings with the FDA.

In terms of performance characteristics, our studies do show minor differences between the two assays. The validation results summarized in Table 1 suggest the BioServ assay to have slightly better

intra-assay precision whereas the ScheBo test is somewhat better in inter-assay performance. The BioServ ELISA exhibits a two-fold improvement in the lower limit of detection compared to the ScheBo (4 vs. 8 $\mu\text{g PE-1/g stool}$ respectively). However, both limits are well below the 100 $\mu\text{g/g}$ medical decision value separating severe and mild pancreatic insufficiency, thus making this difference practically irrelevant.

Linearity studies suggest the ScheBo PE-1 assay to have better linearity throughout the entire AMR than the BioServ assay. Visual inspection of the BioServ ELISA linearity results implicates some underestimation of high PE-1 concentrations. However, because patients with PE-1 values above 200 $\mu\text{g PE-1/g stool}$ are considered healthy, the non-linearity observed at high concentrations is not clinically relevant. What is of greater importance however, is the interval from zero to 200 $\mu\text{g PE-1/g stool}$ that is indicative of abnormal pancreatic function. Within this critical interval, the linearity exhibited by the two assays was essentially equivalent. As indicated in Table 1, the BioServ test did have a slightly better slope than the ScheBo assay. Conversely, the ScheBo test improved somewhat in the intercept (Table 1). Nonetheless, no severe dissimilarities in linearity are evident between the two ELISAs within this important PE-1 concentration interval.

Comparison of the two PE-1 assays by way of a split sample study did demonstrate limited quantitative agreement. We presume this may be caused by the different antibodies used (polyclonal versus monoclonal for the BioServ and ScheBo ELISAs respectively) and the partial digestion of PE-1 in the feces (23,24,31-33). However, as observed in Figure 1 and by the slope and intercept generated by the Deming Regression analysis, neither ELISA demonstrates a consistent trend favoring low or high values over its competitor. This is supported further by nearly identical means of 229 and 226 $\mu\text{g PE-1/g stool}$ generated by the BioServ and ScheBo assays respectively, and by the Bland-Altman Plot analysis shown in Figure 2. Although the difference plot of Figure 2 indicates a substantial difference in PE-1 values for various sample pairs, again there is no indication of a steady or common bias between the two assays. Moreover, these results overall do not reveal any inclination as to which test

more accurately quantifies PE-1. Similar head to head analyses included in a study by Keim et al resemble our results, although that group did conclude the BioServ ELISA to have an approximate 9% increase in sensitivity versus the ScheBo assay (26).

Our results clearly show disagreement between the two ELISAs when comparing the two quantitatively. In addition, studies have suggested that a polyclonal assay may be superior to a monoclonal assay for PE-1 (26, 32), or possibly inferior (34). Nevertheless, what is of utmost importance is how well the PE-1 ELISAs classify patients in terms of pancreatic function, that being normal, mild insufficiency or severe insufficiency.

From the results summarized in Table 2, the agreement in patient classification between the two tests is greater than 90%. Moreover, the small number of disagreements that exist differ by only one classification level. None differ by two levels. Furthermore, these discrepancies in classification are evenly distributed between the two assays, with three individuals classified one level higher in pancreatic function using the SheBo ELISA, and three others classified one level higher utilizing the BioServ assay. Again, this implies that neither ELISA has an overall predisposition over the other in terms of classifying pancreatic function. Taken as a whole, these results demonstrate the BioServ PE-1 ELISA to be at least fundamentally equivalent to the ScheBo PE-1 assay, assuming that the tests are intended to assess pancreatic function with high sensitivity and specificity and not to distinctively measure PE-1 in healthy individuals.

Having established the BioServ PE-1 ELISA as an acceptable substitute for the ScheBo PE-1 test, we performed some data mining studies encompassing results generated using both assays. One aspect we believe that may be of interest to certain clinicians is the PE-1 distribution for clinical specimens particularly for infants, children and young adults. From the ARUP database, we extracted 400 results comprising an ample population of clinical subjects meeting the age criteria of zero to twenty-five. An analysis of the cumulative distribution plot in Figure 3 reveals a fairly dichotomous PE-1 distribution for this age group. As would be expected, the majority or 78% have normal fecal PE-1 concentrations

> 200 µg PE-1/g stool. With another 15% resulting in PE-1 < 100 µg/g and thus categorized a severely pancreatic insufficient, only a minimal 7% remain classified midway as mildly insufficient having values between 100 and 200 µg PE-1/g stool. It is reasonable to presume that the majority of testing was initiated due to a suspected decline in pancreatic activity. Consequently, the data would primarily suggest that in individuals suffering pancreas related illnesses, the deterioration in pancreatic function generally occurs in a relatively short time frame.

Although far from ideal, fecal fat analysis is regularly used for evaluating pancreatic function (22). From the database, we were able to find 44 individuals having had both fecal fat and PE-1 tested within a three month period. Because fecal fat testing cannot distinguish fat malabsorption caused by pancreatic insufficiency from other sources (22), and because fecal fat may be normal in pancreatic insufficient subjects taking exogenous enzyme replacements, we did not expect to find a reasonable correlation. This was confirmed as illustrated in Figure 4. The plot shows that subjects with low PE-1 can have normal or increased fecal fat concentrations. The only potential correlation suggested is for individuals with very high fecal fat (> 7%) having very low fecal PE-1 concentrations. The suggestion however, is limited because of the paucity of data in this region of the graph.

Quantitative fecal fat analysis requires collection of feces for up to three days. The PE-1 test requires only 0.1 g of stool thereby, making specimen collection much more convenient for the patient. Because fecal PE-1 has been shown to have high sensitivity and specificity in diagnosing pancreatic insufficiency (5-11), and the correlation with fecal fat appears minimal, we propose that PE-1 replace fecal fat for such testing.

In summary, the BioServ Diagnostics fecal PE-1 ELISA provides acceptable performance characteristics for assessing pancreatic exocrine function and is a suitable alternative for the previously established ScheBo Biotech PE-1 stool test. Furthermore, both assays are essentially equivalent in terms of classifying patients as normal, or as mildly or severely pancreatic insufficient. We also recommend that fecal PE-1 replace fecal fat analysis for the assessment of pancreatic function.

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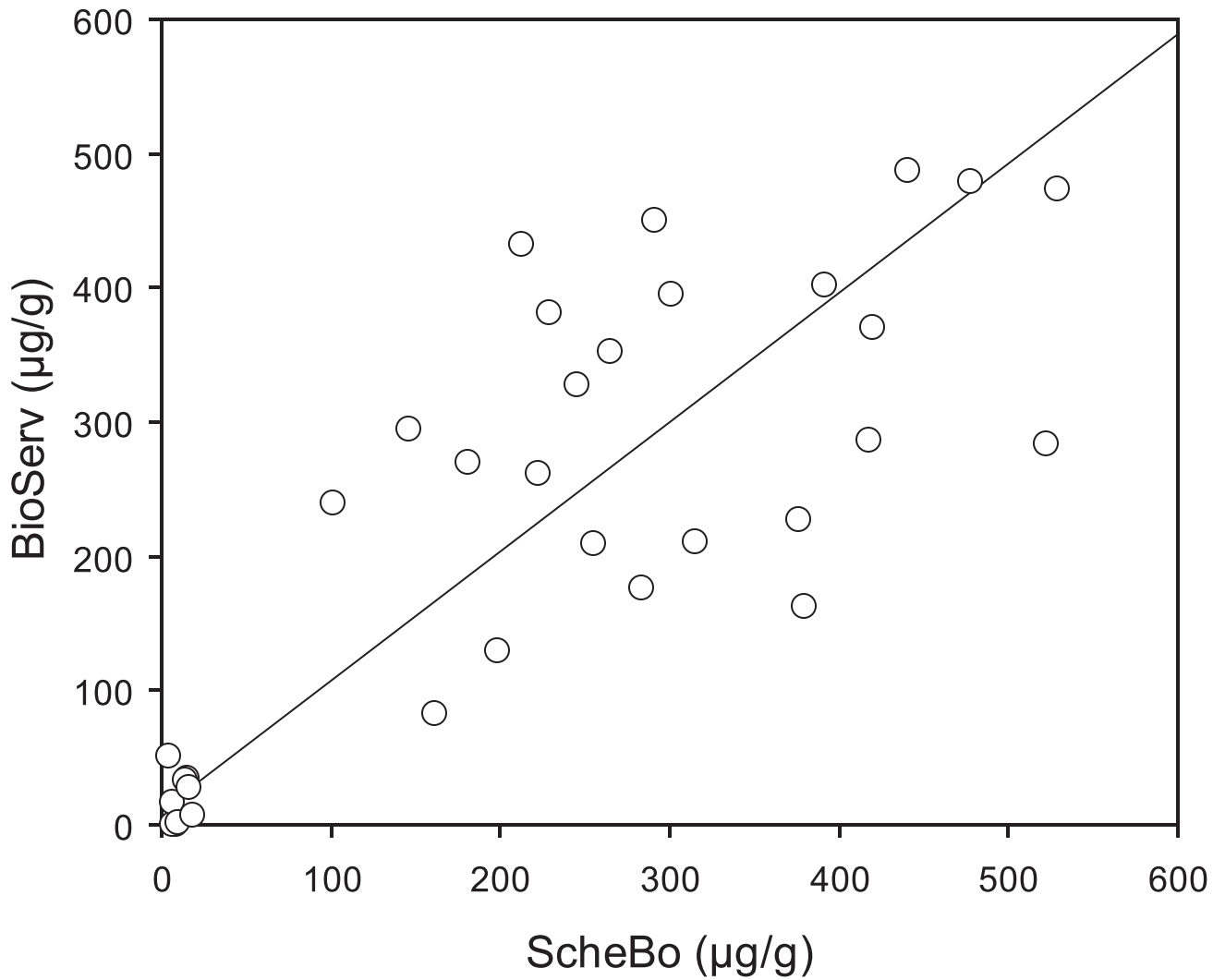
FIGURE LEGENDS

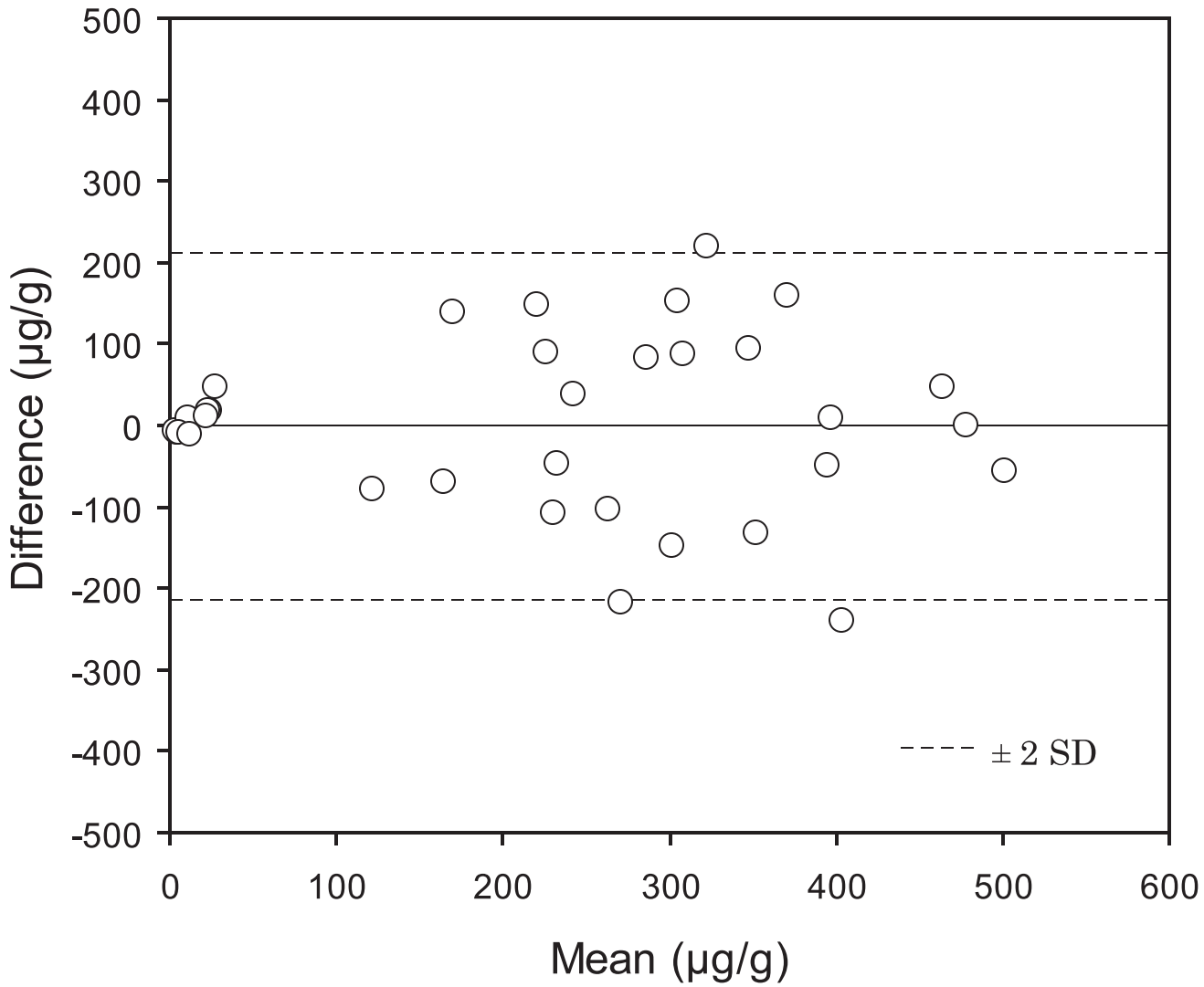
Figure 1. Deming Regression: BioServ versus ScheBo PE-1 ELISAs. Stool samples were extracted and assayed according to each kit manufacturer's instructions. Only samples within or near the analytical measurement range of 500 μg PE-1/g stool were included ($n = 33$). Deming Regression analysis (solid line): $y = 0.9640x + 10.787$, $R^2 = 0.633$.

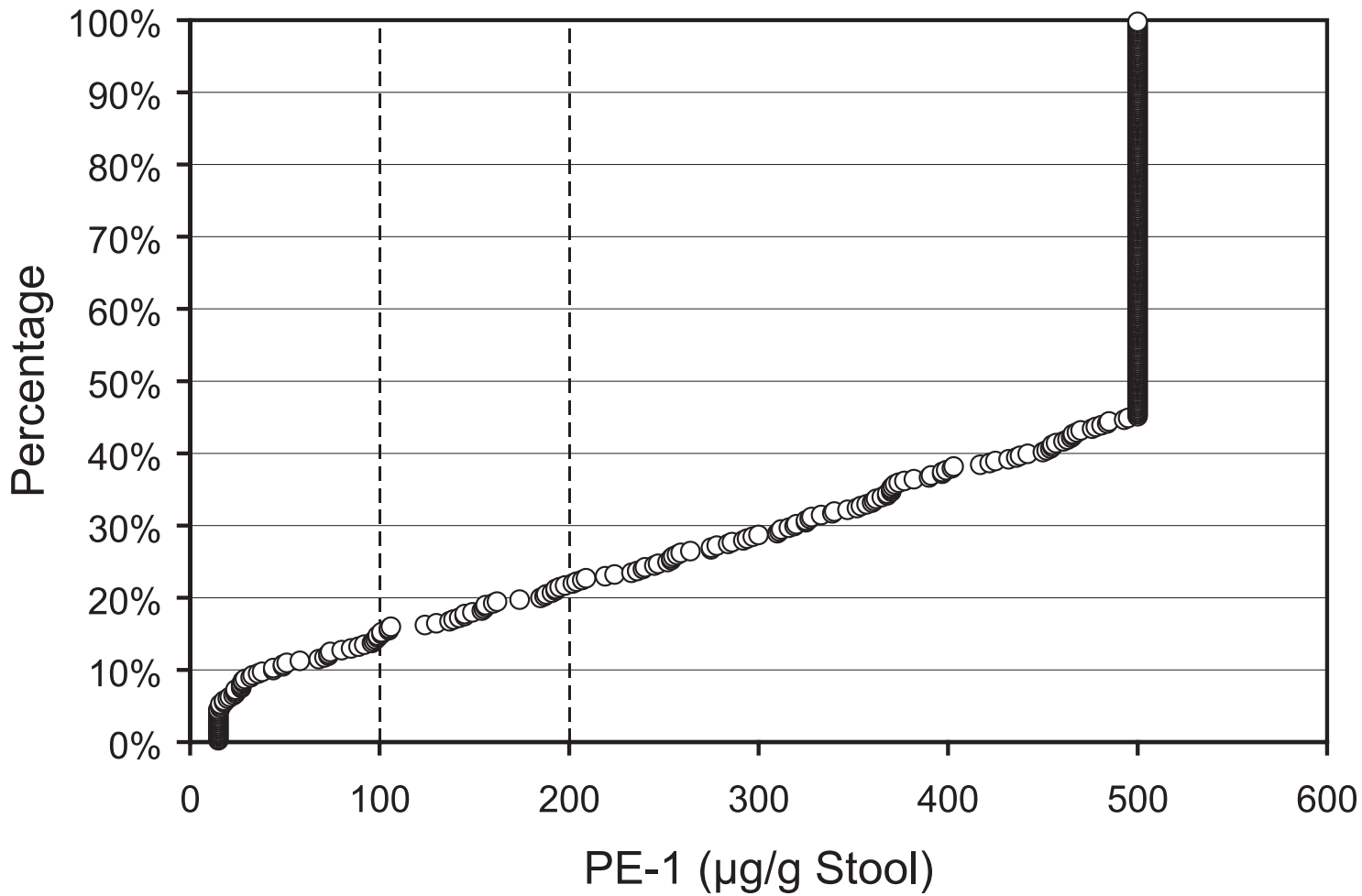
Figure 2. Bland-Altman Plot: BioServ versus ScheBo PE-1 ELISAs. Stool samples were extracted and assayed as instructed by each kit manufacturer. Only samples within or near the analytical measurement range of 500 μg PE-1/g stool were included ($n = 33$). The dashed lines represent two standard deviations of the difference from the means.

Figure 3. PE-1 Distribution of Clinical Specimens Ages 0 to 25. PE-1 test results from July 2003 through November 2004 ($n = 400$). Dashed lines represent the reference interval cutoff values between severe and mild pancreatic insufficiency (100 μg PE-1/g stool), and mild insufficiency and normal values (200 μg PE-1/g stool).

Figure 4. PE-1 versus Fecal Fat. PE-1 and fecal fat results for patients having had both tests within a three month period ($n = 44$). For uniformity, fecal fat results were converted to percentages.







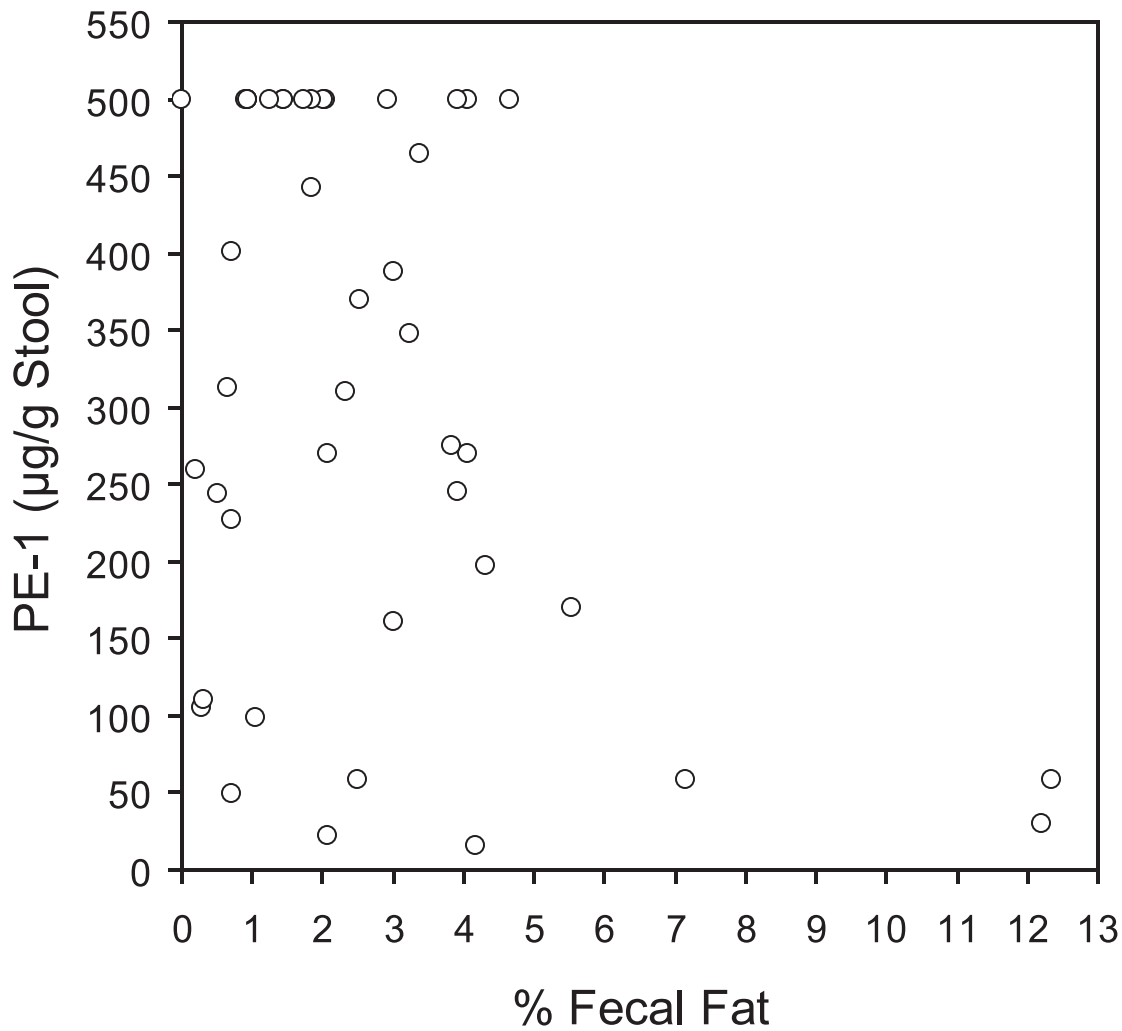


Table 1. Pancreatic Elastase-1 ELISA Performance Characteristics

Assay	Level	Imprecision								Detection				
		Within-run (n = 10)				Between-run (n = 6)				Limit (µg/g)	Linearity (0 to 200 µg/g)			
		Mean PE-1 (µg/g)	Standard Deviation	CV%	n	Mean PE-1 (µg/g)	Standard Deviation	CV%	n		Slope	Intercept	R ²	n
BioServ	I	115	4.3	3.8	10	118	15.6	13.2	6	4	0.987	15.6	0.970	6
	II	204	6.8	3.3		176	16.6	9.4						
	III	361	10.8	3.0		398	33.8	8.5						
ScheBo	I	57	6.1	10.7	10	67	7.0	10.4	10	8	0.971	-5.6	0.997	5
	II	296	13.4	4.5		197	12.4	6.3						
	III	445	17.3	3.9		423	21.9	5.2						

Table 2. Pancreatic Function Classification Comparison

		BioServ		
		Severe	Mild	Normal
ScheBo	Classification ^a			
	Severe	9	0	0
	Mild	1	1	3
Normal	0	2	52	

^aDisagreements in bold type.
