Monoclonal versus Polyclonal ELISA for Assessment of Fecal Elastase Concentration: Pitfalls of a New Assay, Arne Schneider,1*, Benjamin Funk,2 Wolfgang Caspary,1 and Juergen Stein1 (1 Medical Department I and 2 Department of Pediatrics, University Hospital, Frankfurt/Main, Germany; * address correspondence to this author at: Medical Department I, Johann Wolfgang Goethe-University Frankfurt, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main, Germany; fax 49-69-6301-6448, e-mail arne.schneider@em.uni-frankfurt.de)

Exocrine pancreatic insufficiency is a frequent consequence of chronic or severe acute pancreatitis. Assessment of exocrine pancreatic function is commonly performed with stool tests, which have largely replaced the need for invasive function tests. Elastase is excreted by the pancreas and passes through the intestine without significant degradation or inactivation. Consequently, measurement of fecal elastase fulfills the requirements of an almost ideal surrogate marker for pancreatic exocrine function (1–5). The enzyme remains relatively stable in vitro, allowing stool samples to be mailed to a laboratory for analysis.

To date, the assay most widely used for measurement of pancreatic elastase has been a commercially available ELISA with a monoclonal antibody. The assay has been reported to be positive in 93% of patients with severe exocrine pancreatic insufficiency and in 63% of those with mild chronic pancreatitis, and negative in 93%–96% of those without pancreatic insufficiency. In contrast to many other pancreatic function tests, the analysis does not require interruption of oral porcine pancreatic replacement therapy because only the human form of elastase 1 is detected by the assay (1–5).

A new polyclonal antibody test using 2 different polyclonal antisera to human elastase has been reported to be positive in 78% of patients compared with 69% positivity for the monoclonal test in the same patients at a cutoff of 200 μg/g elastase (6). A cutoff of 100 μg/g gave similar sensitivities and specificities for the 2 tests. Binding studies showed that the polyclonal test seems to detect antigens that partly differ from “classic” elastase 1 (7). Simultaneous evaluation of stool specimens showed a tendency for higher values in the polyclonal test, which might cause a higher proportion of false-negative results.

We addressed 2 major questions: Does the polyclonal test specifically detect a human antigen when used to test samples from patients receiving oral porcine enzyme replacement therapy? Does the corresponding fecal antigen of the polyclonal test remain as stable as fecal elastase 1 in stool samples stored under different conditions?

The microtiter plates for the monoclonal elastase test (mET) are coated with 2 monoclonal antibodies that specifically bind elastase 1 in stool specimens (Pancreatic Elastase 1; Schebo Biotech AG). The test is based on an immunoenzymatic method, which we performed according to the manufacturer’s guidelines. The polyclonal elastase test (pET; Elastase 1 ELISA) was purchased from BioServ Diagnostics. This assay uses 2 polyclonal antisera that recognize different antigenic epitopes of pancreatic elastase. The test procedures are comparable with those of the mET.

We measured fecal elastase in stool samples from 27 patients with cystic fibrosis (CF) and 10 patients with normal exocrine pancreatic function. The patients with CF had severely compromised exocrine pancreatic function, and all were receiving oral pancreatic enzyme replacement therapy consisting of 5000–10,000 U of porcine lipase·kg⁻¹·day⁻¹.

Each patient with normal exocrine function provided a stool specimen, which was divided into 4 parts: 1 was tested directly after defecation with both the monoclonal and the polyclonal assays; the other 3 were stored at 4 °C, room temperature (22 °C), or 37 °C. We simultaneously analyzed these 3 portions for elastase with both assays after 24 and 48 h.

Statistical analyses were performed with GraphPad Prism, Ver. 4.01. Comparisons between the elastase concentrations obtained with the mET and the pET were performed with the Wilcoxon test. A P value ≤0.05 was considered significant.

In CF patients receiving oral enzyme replacement therapy, the mET showed pathologically decreased fecal elastase concentrations in all patients and the pET gave normal or near-normal elastase concentrations (Fig. 1). This difference was highly significant [mean (SD), 138 (69) and 4.3 (9.0) μg/g with the pET and mET, respectively; P < 0.0001].

In addition, we analyzed a stool sample from a patient with chronic pancreatitis and severely compromised exocrine pancreatic function with both the mET and pET before and after addition of 100 and 300 U of commercially available porcine lipase (Kreon®; Solvay GmbH).

![Figure 1](image-url)
which had been dissolved in a NaHCO₃ solution (84 g/L NaHCO₃). The elastase concentrations measured with the mET and pET before addition of lipase were 2.2 and 23.2 µg/g, respectively. After the addition of pancreatin, the measured concentrations were 2.8 µg/g (100 U) and 2.9 µg/g (300 U) in the mET vs 66 µg/g (100 U) and 244 µg/g (300 U) in the pET. We also measured fecal elastase in samples from 5 patients with chronic pancreatitis before and during oral enzyme replacement therapy (500–1000 U of porcine lipase · kg⁻¹ · meal⁻¹). Before the initiation of oral enzyme replacement therapy, measured elastase concentrations were 1.3 (0.9) µg/g in the mET and 18.2 (17.5) µg/g in the pET. Whereas the mET result remained low [1.8 (0.9) µg/g] after the initiation of oral enzyme therapy, the elastase concentrations measured by the pET assay increased significantly [62.2 (31.1) µg/g; \( P = 0.03 \)].

Compared with elastase concentrations assessed directly in fresh stool specimens, the measurements performed after 24 and 48 h of storage at different temperatures gave heterogeneous results. There was a tendency for decreasing elastase concentrations in the samples measured after 24 and 48 h of storage at 4 and 37 °C with the mET (Table 1). The results of the pET were significantly higher after 48 h at 4 °C (\( P = 0.004 \)), whereas the other analyses seemed stable irrespective of whether the samples were stored at room temperature or 37 °C.

Although fecal elastase testing has a low sensitivity for the diagnosis of mild exocrine pancreatic insufficiency, the assay reliably predicts clinically relevant degrees of pancreatic insufficiency and is widely used in clinical medicine (7). In contrast to fecal chymotrypsin, the monoclonal elastase 1 assay does not require interruption of oral enzyme replacement therapy because commercially available enzyme compounds do not interfere with the test (9). With regard to this point, the widely different results obtained with the well-established mET compared with the relatively new pET ELISA among our patients with CF are a disappointing finding. We can proceed from the assumption that the CF patients studied suffered from severely compromised exocrine pancreatic insufficiency because the disease inevitably leads to the destruction of virtually all pancreatic tissue: ~60% of neonates diagnosed with CF already suffer from pancreatic insufficiency (10). This proportion increases to 92% during the first year of life (11). To maintain adequate nutrition, patients require oral pancreatic enzyme replacement therapy. As a consequence, an explanation for the substantial difference between the elastase tests is that the polyclonal assay is influenced by the oral pancreatic supplement, which consists of capsules containing porcine pancreatin, lipase, amylase, and proteases. The finding of measured concentrations >100 µg/g elastase in the pET in the majority of CF patients may reflect adequate high-dose oral enzyme substitution.

A recently published study concluded that the antibodies used in the pET assay detect antigens different from elastase 1 (7). In a PubMed search, we found no additional protein-binding studies elucidating the interaction of pET antibodies with fecal antigens. Accordingly, our findings support the thesis that the pET assay detects antigens that are different from elastase 1 but are probably contained in oral pancreatic enzyme capsules. This has also been shown in our own laboratory by simple addition of dissolved pancreatin from commercially available enzyme capsules to fecal specimens, which produced a proportional increase in the elastase concentration detected by the pET. The same was true for the comparative analysis of stool samples from patients with chronic pancreatitis before and during “in vivo” substitution of enzymes. The fact that the pET generally gives higher results than the mET further supports the concept of a different antigenic specificity. For this reason, we cannot recommend that the pET be used to monitor patients receiving oral enzyme replacement therapy, which is a major drawback for the clinical application of the pET.

On the other hand, the antigenic substrate in the pET seems to be equally stable in relation to elastase 1. Only elastase concentrations determined with the pET in stools stored at 4 °C for 48 h were significantly higher than directly measured concentrations. Although both pET and mET assessments were performed on stool samples stored in continuously closed cups, partial evaporation with consecutive concentration of the substrate cannot be excluded.

If we take into consideration the different aspects of fecal elastase testing highlighted in this study, a lack of specificity of the pET assay remains the crucial problem. We therefore support the proposal of renaming the polyclonal “elastase 1” ELISA because the test detects a molecule different from elastase 1 (7). Further studies should evaluate the effects of porcine enzymes on the results of the pET assay in patients with normal and mildly to moderately decreased exocrine pancreatic function. Additionally, the true antigen detected with the pET assay should be characterized to reliably define the role of this new assay in the diagnosis of exocrine pancreatic insufficiency.

### Table 1. Fecal elastase concentrations in 10 stool samples assayed by mET and pET after 24 and 48 h of storage at 4 °C, room temperature (22 °C), or 37 °C.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Mean (SD) of direct measurement, µg/g</th>
<th>Mean (SD) measured elastase, µg/g</th>
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<tbody>
<tr>
<td></td>
<td>Storage at</td>
<td>24 h</td>
</tr>
<tr>
<td>mET</td>
<td>434 (122)</td>
<td>4 °C (428 (110))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 °C (454 (157))</td>
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<tr>
<td></td>
<td></td>
<td>37 °C (436 (161))</td>
</tr>
<tr>
<td>pET</td>
<td>544 (93)</td>
<td>4 °C (561 (118))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 °C (538 (110))</td>
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<tr>
<td></td>
<td></td>
<td>37 °C (500 (146))</td>
</tr>
</tbody>
</table>

*\( P = 0.004 \) vs directly measured elastase.
References


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