Comparison of Non-Diglyceride- and Diglyceride-Based Assays for Pancreatic Lipase Activity

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Pancreatic lipase is produced and stored in pancreatic acinar cells, and is normally secreted into the duct system. In disorders that cause pancreatic damage, pancreatic lipase enters the circulation, and serum lipase activity becomes useful in the diagnosis and evaluation of pancreatitis. During the last decade, many lipase assays have used the Imamura method, in which a series of enzymatic reactions utilizing a diglyceride substrate leads to the formation of a colorimetrically detected product. Historically, this method has been prone to significant interference from various substances, including glycerol itself. In the light of these limitations, we evaluated an automated enzymatic rate assay that uses a non-diglyceride-based, pancreatic lipase-specific substrate. Precision, linearity, and potential interference were assessed, and when compared to the Imamura method, the non-diglyceride-based assay exhibited a slope of

0.475, y-intercept of 15.89, r-value of 0.9516, and $S_{y,x}$ of 12.96. Similar results were also observed when the two assays were compared using samples with markedly elevated creatinine levels. Between-day coefficients of variance (CVs) ranged from 5.0% to 5.5%, which compared well with the diglyceride-based method, and linearity spanned a range of 3–156 U/L. Evaluation of over 2,000 patient results collected during a 6-month period suggested that the manufacturer's upper reference limit of 51 U/L may be too conservative. No significant interference was identified with bilirubin, triglyceride, or glycerol itself, and significant interference from hemoglobin was observed only at concentrations of 116 mg/dL or greater. As a result, the non-diglyceride-based method provides an acceptable alternative for the routine laboratory measurement of lipase activity. *J. Clin. Lab. Anal.* 16:52–55, 2002. © 2002 Wiley-Liss, Inc.

Key words: lipase; pancreas; pancreatitis; triglyceride

INTRODUCTION

Lipase is an enzyme produced in the pancreas, gastrointestinal tract, and (to a lesser extent) other tissues (1). The concentration of lipase in pancreatic tissue, where it is produced and stored in acinar cells, is normally 20 times the serum lipase level and 30–40 times higher than in the intestine (1). More than 99% of pancreatic lipase is secreted into the ductal system, with less than 1% normally leaking into the general circulation (1). However, inflammatory and obstructive disorders of the pancreas result in excess extravasation of lipase into the circulation, where it can be detected and quantitated (1,2).

Prior to the last decade, serum lipase activity assays suffered from poor precision, and total serum amylase activity remained the favored marker of pancreatic dysfunction (2). With the development of rapid and automated enzyme assays for lipase, serum lipase activity has become recognized as a sensitive and specific test that can assist in the diagnosis of acute pancreatitis or other pancreatic disorders (1–4). During the last decade, many commonly used assays for lipase activ-

ity have utilized diglyceride-based substrates and a complex reaction scheme first described by Imamura (4–7) (Fig. 1). While these assays are reliable in most cases, they are subject to interference from glycerol and cannot conveniently be run on instruments on which triglyceride assays are routinely performed (6).

However, not all assays rely on a diglyceride-based substrate. Notable exceptions include the ACA assay (Dade Behring, Deerfield, IL), which uses a triolein substrate, and the Roche/Hitachi Lipase Assay (Roche Diagnostics Corp., Indianapolis, IN), which utilizes two coupled reactions and a non-diglyceride substrate to form methylresorufin. Recently, Beckman Coulter, Inc. (Fullerton, CA) marketed its own non-diglyceride-based lipase reagent (LIP) for the quantitative

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LIPA Assay Reaction Scheme

Fig. 1. Imamura method. This schematic shows the reaction scheme utilized in the LIPA assay involving a 2-monoglyceride lipase.

determination of serum or plasma lipase activity on SYNCHRON LX Systems in random access mode. This assay is based on the same principle as the Roche assay, and the resulting chromogenic end product is quantitated spectrophotometrically. In this reaction scheme, which is shown in Fig. 2, the rate of product formation is proportional to the lipase activity (8,9). This assay has a potential advantage over Beckman Coulter's previous diglyceride-based assay because interference from glycerol is eliminated, and the assay can be performed on the same LX-20 instrument that measures triglyceride levels, without performing complex washing procedures. In this study, we assessed the LIP assay and compared it with Beckman Coulter's diglyceride-based (LIPA) assay (10).

MATERIALS AND METHODS

The LIP assay consists of an automated colorimetric enzyme rate assay that can measure lipase activity in serum or heparinized plasma. The assay was developed for SYNCHRON LX systems in random access mode in conjunction with the SYNCHRON Enzyme Validator Set. It consists of reagents necessary for the coupled chemical reactions (Fig. 2), including the pancreatic lipase-specific, non-diglyceride substrate (1,2-o-lauryl-rac-glycero-3-glutaric acid-(6'-

LIP Assay Reaction Scheme

Fig. 2. Panteghini method. The reaction scheme utilized in the LIP assay involves a pancreatic lipase-specific substrate.

methylresorufin)-ester). The rate of formation of the final product, methylresorufin, is measured colorimetrically at 560 nm, and the rate is directly proportional to the lipase activity in the specimen (9).

In this study, all lipase measurements were performed on SYNCHRON LX-20 instruments (10). For the method comparison we performed lipase measurements on 100 serum samples from hospital patients using the LIP and LIPA assays on each specimen. We also performed lipase measurements on 52 serum samples from hospitalized patients with renal failure (as indicated by creatinine levels > 3.0 mg/dl) using both assays. For linearity studies, a high patient sample (156 U/L) was diluted with physiological saline, and lipase activity was measured at the following dilutions: 1:1, 1:2, 1:3.8, 1:8.3, 1:16.6, and 1:50. Each sample was run in quadruplicate and the mean and standard deviation (SD) were plotted against expected values. Precision studies were performed using low- and high-level controls. Within-run precision was performed on 20 consecutive replicates, whereas between-day precision was calculated using data from 20 runs at both levels.

To evaluate the range of lipase activity as determined by the LIP assay in our patient population, we collected 2,017 consecutive LIP lipase results over a period of 6 months and plotted these results as a distribution graph, with intervals spanning 10 U/L of lipase activity. Interference studies were performed using three serum pools spiked with increasing amounts of bilirubin (Sigma, St. Louis, MO), hemoglobin (from a hemolysate prepared from osmotically shocked heparinized blood), triglyceride (Liposyn, a commercial fat emulsion manufactured by Abbott Laboratories, Abbott Park, IL), and glycerol (Sigma). Lipase activities were compared to the original serum pool plus vehicle, and the percent change was calculated for each concentration. Six to nine concentrations each of bilirubin, hemoglobin, triglyceride, and glycerol were tested at concentrations ranging from 1.0–55.4 mg/dl, 8–925 mg/L, 147–2,261 mg/dl, and 25–300 mg/dL, respectively.

RESULTS

The results obtained using the LIP assay demonstrated acceptable correlation with the LIPA method, with an *r*-value of 0.9516, a *y*-intercept of 15.89 U/L, and a slope of 0.475 (Fig. 3). The positive intercept suggests that the LIP assay has a possible negative bias with respect to the LIPA method. Similar results were obtained when the two assays were compared using serum from patients with renal failure (*r*-value: 0.9570; *y*-intercept: 9.33 U/L; slope: 0.549) (Fig. 3). Based on the results obtained with serial dilutions of a high patient sample (156 U/L), the LIP assay demonstrated good linearity ($S_{y,x} = 3.6$) of 3–156 U/L (Fig. 4). The manufacturer's recommended limit of detection is 200 U/L.

Between-day precision was assessed at two different levels: 35 U/L and 110 U/L. The low-level control yielded a

Fig. 3. Comparison of the LIP assay with the LIPA assay. Left panel: Levels of serum lipase activity in 100 patients were determined by the LIP lipase assay and plotted against those obtained with the LIPA method. The y-intercept is 15.89, slope 0.475, r-value 0.9516, and $S_{y,x}$ 12.96. Right panel:

A similar correlation was performed for 50 patients with renal disease (creatinine > 3.0 mg/dl). The y-intercept is 9.33, slope 0.549, r-value 0.9570, and $S_{y,x}$ 10.99.

mean of 36.2 U/L with a coefficient of variation (CV) of 5.4%, while the high-level control yielded a mean of 106.4 U/L and a CV of 5.3%. These results are well below the manufacturer's recommended highest acceptable between-run CV of 10.0%.

A total of 2,017 consecutive lipase activities obtained over a period of 6 months using the LIP assay on hospital patients were plotted on a distribution graph using intervals of 10 U/L of lipase activity (Fig. 4). The resulting graph shows declining relative percentages up to a lipase activity of 100 U/L, suggesting that the manufacturer's suggested upper reference limit may be too conservative for an in-patient population.

Interference studies using the LIP assay demonstrated a <10% difference in measured lipase activity for the concentrations of bilirubin, triglyceride, and glycerol tested. Hemoglobin interference became significant at 116 mg/dL with a negative bias of 12.0%. In comparison, the LIPA assay exhibited a negative bias of 15.5% at 28.8 mg/dL of bilirubin, and complete suppression of test results when hemo-

globin and glycerol levels exceeded 116 mg/dL and 150 mg/dL, respectively.

DISCUSSION

Historically, serum enzyme assays for the diagnosis and evaluation of pancreatic disease have exhibited variable results. In the last decade, enzyme-rate-based measurement of serum lipase activity has been the method of choice for the diagnosis and follow-up of pancreatitis and other pancreatic disorders, and has been shown to be a sensitive and specific indicator of acute pancreatic damage (1–3). A popular enzyme-rate method, described by Imamura et al. (5), utilizes a diglyceride-based substrate. Assays currently using this method include the Advia Lipase Assay (Bayer Corp., Tarrytown, NY), the Vitros LIPA Slides (Johnson and Johnson Clinical Diagnostics, Inc., Rochester, NY), and the Abbott Aeroset Lipase Assay (Abbot Laboratories). The ACA Assay

Fig. 4. LIP assay linearity and patient data. Left panel: Linearity was examined using serial dilutions of a patient sample (156 U/L). The $S_{y,x}$ = 3.6, y-intercept is 6.9, and the claimed linearity of the test is 200 U/L. Y-error bars representing the SD are too small to be seen. Right panel: 2,017 con-

secutive lipase activities obtained using the LIP assay on hospital patients over a 6-month period were plotted on a distribution graph using intervals of 10 U/L of lipase activity.

(Dade Behring) utilizes a triolein substrate. Unfortunately, diglyceride-based serum lipase activity assays are subject to interference from glycerol and can not conveniently be used on instruments on which triglyceride levels are measured.

To circumvent this problem, the Roche Diagnostics Corporation has for several years marketed a non-diglyceride-based reaction scheme. Following the example set by Roche, Beckman Coulter has introduced its own assay, which is claimed to suffer no significant interference from bilirubin, hemoglobin, or lipemia. This is in contrast to Beckman Coulter's claim that its older, diglyceride-based LIPA assay is subject to a significant negative bias from bilirubin and glycerol, and its caution against using the LIPA assay on an instrument on which esterase-containing assays are normally performed.

Our interference results generally support Beckman Coulter's stated claims for the LIP assay, although some negative interference with hemoglobin was observed. Notably, Beckman Coulter did not report glycerol interference data. Our own data show the LIP assay suffers no significant interference from glycerol, in marked contrast to the LIPA assay, which exhibited significant negative interference and complete suppression of test results at glycerol levels exceeding 150 mg/dL.

When sera from renal failure patients (creatinine > 3.0 mg/dl) were evaluated, the LIP assay correlated well with the LIPA assay. This suggests that increased lipase activity measurements in patients with renal failure are real and are not a result of interference from nonspecific esterases, which may be elevated in renal failure (11).

Because the LIP assay exhibited a notable negative bias compared to the LIPA assay, we proceeded to examine lipase activity data from our institution over a 6-month period using the LIP assay. The evaluation of 2,017 consecutive assays from our hospital population suggests the manufacturer's upper limit of normal (51 U/L) may be too low for our patient population. The manufacturer's normal range of 22–51 U/L was determined by measuring lipase activity in 125 normal individuals (outliers were excluded) and including the non-parametric 95th percentile as the normal range. The mean was determined to be 32.5 U/L, with an SD of 8.3 U/L (12). No information was given as to how many outlying data points

were discarded. Our own data indicate a declining trend in lipase activity up to 100 U/L, after which a second small peak is identified; this may represent a more meaningful upper reference limit for hospitalized patients.

In addition to the improved interference profile, the Beckman Coulter LIP assay offers additional significant advantages over the LIPA assay. The reaction scheme is simpler, consisting of two reactions as opposed to five. Furthermore, the use of a non-diglyceride substrate eliminates the necessity of complex washing procedures or having to perform LIP and triglyceride assays on separate instruments. As a result, we have replaced the LIPA assay with the LIP assay in our own laboratory.

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