assay measures cholesterol associated with IDL and LDL but not with Lp(a) particles. The significant negative bias observed may be attributed to suboptimal assay calibration. After readjusting the calibration, we found a close relationship between LDL-C Plus and LDL_{BQ} values, and LDL-C Plus became a reliable alternative to beta-quantification and a better approach for LDL-C measurement than the Friedewald formula.

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Effect of Specimen Anticoagulant and Storage on Measurement of Serum and Plasma Fatty Acid Ethyl Ester Concentrations, *Raneem O. Salem, Joanne E. Cluette-Brown, Ali Hasaba, and Michael Laposata** (Division of Laboratory Medicine, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; * Address correspondence to this author at: Room 235, Gray Bldg., Massachusetts General Hospital, Boston, MA 02114; fax 617-726-3256, e-mail mlaposata@partners.org)

Fatty acid ethyl esters (FAEEs) are cytotoxic nonoxidative metabolites of ethanol that are produced by esterification of alcohol and fatty acids *(1–3)*. After ethanol intake, FAEEs are found mainly in the liver, pancreas, heart, and brain, which are the major organs adversely affected by ethanol intake *(4, 5)*. FAEEs are detectable in the blood for up to 24 h after consumption of ethanol to at least 600 mg/L *(6–8)*, making the presence of FAEEs in blood a useful marker for ethanol intake. This investigation presents the results of studies on the effects of collection tube, storage time, and storage temperature on FAEE concentrations in blood, with the goal of creating a reliable clinical assay for serum and plasma FAEE quantification.

Four volunteers participated in this study. Each subject was given a weight-adjusted amount of 100-proof vodka mixed with fruit juice in a 1:3 ratio *(8)*. The vodka-juice beverage was divided into nine equal aliquots, which were administered every 10 min over a 90-min time period. After the last aliquot was drunk, blood samples were collected in four different 5-mL Vacutainer Tubes from each subject to assess the influence of the collection tube on the blood FAEE concentration. The tubes included a red-top tube with no anticoagulant, a purple-top tube with 150 g/L EDTA, a green-top tube with 72 USP units of heparin, and a blue-top tube with 32 g/L sodium citrate. The tubes were kept on ice and processed within 30 min. FAEEs were then isolated and quantified as described below. To study the effect of storage time and temperature on FAEE concentrations in whole blood, 40 mL of blood was collected from each of the same four subjects in red-top vacuum tubes (no anticoagulant). The 40 mL of blood was divided into two equal aliquots that were incubated at 25 and 37 °C for 0, 2, 4, 6, 24, and 48 h. FAEEs were then isolated from the samples. To accomplish this, serum or plasma was first separated from the blood cells by centrifugation at 3420*g* at 4 °C for 20 min. FAEEs were then isolated using an acetone-hexane (2:8 by volume) extraction followed by solid-phase extraction *(9)*, and measured using gas chromatography–mass spectrometry as described previously *(10)*. The within-assay CV for the entire method was 17%. The CVs for the individual stages were as follows: FAEE isolation via liquid extraction, ~13%; solid-phase extraction, ~9.7%; and quantification by gas chromatography–mass spectrometry, ~2.2%.

The effect of the collection tube on the amounts of FAEE in plasma or serum is shown in Table 1. For the four

Table 1. Relative amounts of FAEE detected in different blood collection tubes.

Tube top	Contents	FAEE (mean ± SE),[a] nmol/L
Red	No anticoagulant	1712 ± 385
Purple	EDTA	624 ± 60
Green	Heparin	2033 ± 1064
Blue	Sodium citrate	1994 ± 33

[a] n = 4.

volunteers, the mean concentrations of FAEE in the sodium citrate tube, the heparin tube, and the anticoagulant-free tube were similar. However, the EDTA vacuum tube showed a lower FAEE concentration than the others. We speculate that the extensive chelation of calcium by EDTA contributed to this observation in some way. The mean FAEE concentrations in plasma in the sodium citrate and heparin tubes, and in serum in the anticoagulant-free tubes, were more than twice the mean plasma FAEE concentration in the EDTA tubes. The variability was least for sodium citrate tubes. This higher precision suggests the use of 32 g/L sodium citrate as the preferred anticoagulant.

Storage time and temperature also affected FAEE concentrations in serum. The FAEE concentration increased over time when incubated as whole blood at 25 and 37 °C. As shown in Fig. 1, there was a rapid increase in FAEE concentrations at 37 °C from 2273 ± 507 nmol/L (mean ± SE) at time 0 to 4826 ± 245 nmol/L (mean ± SE) 48 h after blood collection. At 25 °C, there was no change in the FAEE concentration after up to 4 h of incubation: 1712 ± 385 nmol/L at time 0 and 1766 ± 486 nmol/L at 4 h (mean ± SE for both). However, there was an increase to 4750 ± 875 nmol/L (mean ± SE) with storage at 25 °C for 48 h. There was less variability, in general, for the samples stored at 37 °C, but this higher precision is not likely to have a biological basis.

The in vitro formation of FAEEs, which is fastest at 37 °C, is most likely the result of enzyme-mediated FAEE synthesis using the residual ethanol in the specimen collection tubes. Ethanol remains in the blood for up to 6 h. White bloods cells, platelets, and to a lesser extent, red blood cells, have been shown to have FAEE synthase activity *(11)*. A previously published study from our laboratory with plasma and serum samples from individuals in a clinical trial involving ethanol ingestion showed no changes in FAEE concentration after 2 days of storage at −4 or −80 °C *(12)*. Taken together, the results of the current storage experiments and our previously reported findings indicate a need for removal of the plasma or serum from the cells within 4 h at room temperature, with freezing of plasma or serum at −4 °C or −80 °C if longer periods of time are required before analysis of the specimen. In addition, samples should not be collected for FAEE analysis in EDTA-containing vacuum tubes.

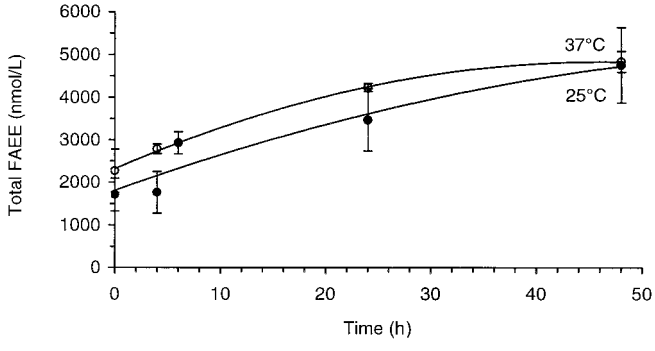

Fig. 1. Effect of time on serum FAEE concentrations at 25 and 37 °C.

Blood samples were collected from four healthy subjects and then incubated for different times before collection of serum. The sera were separated and processed for FAEE measurements as described in the text. Each point represents the mean ± SE (*bars*); n = 4/time point. The differences in mean values are not statistically significant for each time point at 25 vs 37 °C. However, the values at 25 °C at time 0 are different from those at 48 h (P = 0.01), and they approach statistical significance from those at 24 h (P = 0.07). The values at 37 °C at time 0 are different from those at 24 and 48 h (P = 0.05 and P = 0.04, respectively).

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Improved Gas Chromatography–Mass Spectrometry Method for Simultaneous Identification and Quantification of Opiates in Urine as Propionyl and Oxime Derivatives, *Larry A. Broussard,[1*] Lance C. Presley,[2] Mike Tanous,[3] and Cecelia Queen[3]* ([1] Department of Clinical Laboratory Sciences, Louisiana State University Health Sciences Center, New Orleans, LA 70112-2262; [2] LabOne, Inc., Lenexa, KS 66219-9752; [3] LabCorp, Memphis, TN 38118; * author for correspondence: fax 504-568-6761, e-mail lbrous@lsuhsc.edu)

Several authors have reviewed existing methods *(1–9)* or presented new techniques *(6–11)* for the analysis and separation of codeine, morphine, and the keto-opiates