enzymatic assay for ethylene glycol, however, has not been previously documented. The interference may not have been appreciated in previous evaluations of the assay because test compounds, such as lactate and alcohols, were added to normal serum, not sera from critically ill patients [2]. To evaluate the performance of the enzymatic assays for ethylene glycol and ethanol in hospitalized patients with abnormal serum chemistry, we added d,l-lactic acid to two random serum samples with markedly increased LD and performed the enzymatic assays (Table 1). Lactate per se does not interfere in the assay; however, the concomitant presence of LD under the proper assay conditions produces NADH, resulting in substantial false-positive values in the enzymatic assays for ethylene glycol and ethanol. Given their correlation with the concentrations of LD and lactic acid, these false-positive values may be due to the LD-catalyzed conversion of serum lactate and reagent NAD + to pyruvate and NADH.

To support this hypothesis, we reconstituted normal serum with increasing concentrations of LD-5 purified from human placenta (Sigma Chemical Co., St. Louis, MO) in the presence of 0, 10, or 50 mmol/L dl-lactic acid (Sigma). The ethylene glycol enzymatic assay gave falsely positive results when LD serum activity was >3000 U/L, as did the ethanol enzymatic assay when LD serum activity was >2000 U/L (Fig. 1). The interference was most pronounced when the concentration of dl-lactate exceeded 10 mmol/L. These data, however, should not be strictly extrapolated to the clinical setting because the contribution to total LD activity in this experiment was due solely to LD-5, and the concentration of lactate consisted of approximately equal amounts of l- and d-isomers. In hospitalized patients, increased LD activity may be due primarily to the contribution of a different isoenzyme or of more than one isoenzyme, and increased lactate concentration will most probably be the l-isomer. However, the data obtained from three hospitalized patients with increased serum LD activity (Table 1) support a clinically relevant guideline that significant false-positive results occur when the LD serum activity is at least 12-fold greater than the upper limit of the reference interval, and lactate is concomitantly at least 10-fold greater than the upper limit of its reference interval.

High concentrations of lactate and LD in sera from critically ill patients interfered in the enzymatic assay for ethylene glycol by increasing the production of NADH. Although lactate added to normal sera does not interfere with the assay, results should be interpreted cautiously in samples with high LD and lactate concentrations. Other interferences reported for the ethylene glycol enzymatic assay include glyceraldehyde and glycerol, which can be oxidized by the enzyme glycerol dehydrogenase [2]. The enzymatic assay is otherwise very specific, and other compounds, such as lactate and alcohols, were added to normal serum and were not interfering with interpretation. Ethylene glycol poisoning of previously healthy individuals may result in lactic acidosis but will not typically result in increased LD. However, this analytical interference becomes clinically relevant in cases of suspected ethylene glycol poisoning of individuals with hepatic, renal, or cardiac disease who may manifest both lactic acidosis and increased serum LD. Positive results in the enzymatic assay for ethylene glycol in such cases should be confirmed with a different method such as gas chromatography.

We thank Brian Gilmore for expert technical assistance.

References


According to the recommendations of the National Cholesterol Education Program Adult Treatment Panel, low-density lipoprotein cholesterol (LDL-cholesterol) should be used for screening as a primary treatment criterion for patients with increased total cholesterol concentrations [1]. This makes the need for accurate measurements of LDL-cholesterol a national public health imperative. The use of the Friedewald equation is attractive, but its accuracy is in doubt in plasma samples with triglyceride (TG) concentrations >4.5 mmol/L (400 mg/dL) [2-7]. A decennium ago we found the Friedewald equation to be accurate up to a TG concentration of 8 mmol/L [8]. Assuming that similar plasma samples were analyzed, these findings raise questions about the quality of the methods used in other laboratories, especially in the analysis of hypertriglyceridemic samples. The disposal of a direct LDL-cholesterol method not interfered with by hypertriglyceridemia [9] prompted us to evaluate its accuracy in hyperlipidemic samples, including a selection with familial dysbetalipoproteinemia (FD). We hoped that this reevaluation could shed more light on our previously reported claim concerning the accuracy of the Friedewald calculation compared with that of the reference method [8].

Overnight fasted blood samples from healthy persons and from patients with various types of hyperlipoproteinemia were drawn into Vacutainer Tubes containing EDTA (BectonDickinson, Meylan Cedex, France). Plasma samples were analyzed fresh. If sufficient amounts of plasma were available, two aliquots were stored at -80°C for >3 months; one was supplied with saccharose (final concentration 6 g/L). Patients were classified into the different phenotypes according to the criteria of Fredrickson et al. [10] with cutoff limits for plasma cholesterol, TG, and LDL-cholesterol of 6.5, 2.0, and 4.6 mmol/L, respectively. Plasma samples with lipemia, especially those from subjects with FD, were preferentially included. This means that the frequency of samples with FD was considerably higher than in the healthy population. The 217 fresh plasma samples analyzed included, by selection, 63 with a plasma TG concentration >4.5 mmol/L, among which were 31 with a plasma TG >8.0 mmol/L; 11 had a VLDL-cholesterol/plasma TG ratio >0.69
samples of the subjects studied. We used a calibrating serum for statistical results: mean \( v \), 4.01 ± 1.98 mmol/L; mean \( y \), 3.81 ± 1.98 mmol/L.}

VLDL-chol is dependent on age, sex, and sociodemographic characteristics of the supplier, it was found to be essential although the vortex-mixing step was not mentioned in the procedure. I, the reference method was vortex-mixed and assayed for cholesterol. All other samples were also analyzed for total plasma cholesterol. We applied the various methods for determining LDL-cholesterol in 217 fresh plasma samples with TG concentrations ranging from 0.41 to 50.1 mmol/L, including 31 samples with a concentration >8.0 mmol/L and 11 samples of subjects with obligate FD with VLDL-cholesterol/TG ratio >0.69. The results obtained with the LDL-direct method agreed better with the reference method than the estimated LDL-cholesterol values (correlation coefficients: 0.94 vs 0.85, \( S_{xy} \) values 0.34 vs 0.55, respectively). However, after exclusion of the samples with plasma TG >8.0 mmol/L and the samples of the patients with FD (all having a VLDL-cholesterol/plasma TG ratio >0.69), the results obtained with the Friedewald equation correlated very well with the reference method, similarly as those obtained with the LDL-direct method (n = 115) (correlation coefficients >0.97, \( S_{xy} \) values <0.21, regression equations by Passing and Bablock analysis different from \( y = x \)). These results are in agreement with the dilution factors reported previously [8]. Results obtained for the non-FD plasma samples with TG concentrations between 4 and 8 mmol/L were explicitly similar to the general mean results data not shown). These findings can be explained by the relative constancy of the ratio VLDL-cholesterol/plasma TG in the plasma samples with TG concentrations up to 14 mmol/L (Fig. 1). Thus, we did not obtain evidence for a change in the chemical composition of VLDL-cholesterol at increasing plasma TG values.

In the non-FD samples with TG <8.0 mmol/L in which the Friedewald approach gave accurate values, intermediate precision of the Friedewald–ultracentrifugation comparison and the direct LDL-cholesterol ultracentrifugation comparison were similar (\( S_{xy} \) 0.17 vs 0.21, respectively).

A negative bias of ~11% was obtained with the direct method (y) after prior storage of the plasma samples at –80°C compared with the reference method (x); mean x, 4.12 ± 1.98 mmol/L; mean y, 3.58 ± 1.75 mmol/L. Linear regression equation: \( y = 0.88x - 0.02 \), correlation coefficient \( r = 0.93 \), \( S_{xy} = 0.35 \), n = 110. Apparently, some of the LDL was retained in the column. Addition of 6 g of saccharose per liter of plasma before freezing prevented this, as could be derived from the statistical results: mean x, 4.01 ± 1.98 mmol/L; mean y, 3.81 ± 1.75 mmol/L.
the drawing of conclusions concerning the optimal estimation
ported by the results of McNamara et al.
port of hypertriglyceridemic plasma samples are analyzed. This is sup­
we observed, independent of the plasma TG concentration. Our
ported by the relatively constant estimate for VLDL-chol that
observations suggest that in other laboratories one of the
TG (in mmol/L) as an approach. However, even in the non-FD
samples with a plasma TG concentration of 8 to 14 mmol/L,
that the analysis of fasting plasma samples in our study merely
the reference value did not differ from 1.0 (Passing and Bablock
TG (mmol/L), but also in samples with TG concentrations between
plasma samples. Chylomicrons present in nonfasting plasma are
thought to result in too low LDL-chol values because VLDL-
tion in our hands could be due to the fact that we analyzed fasted
plasma samples. Chylomicrons present in nonfasting plasma are
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the direct LDL-method for proper phenotyping of strongly
hyperlipidemic samples. Considering the fact that the measured
plasma triglyceride concentration also gives insight into the
LDL-subfraction pattern
It is also possible, as already suggested by Friedewald et al. [20],
that the reference method is biased or imprecise when hyper­
triglyceridemic plasma samples are analyzed. To prevent this we
determined VLDL-chol directly, whereas most other laborato­
ries determine VLDL-chol indirectly as the difference of total
cholesterol and the cholesterol present in the d >1.006 kg/L
fraction. The latter procedure can result in highly variable
VLDL-chol concentrations involving imprecise cholesterol
analysis. In the same way, relatively large CVs of the methods
used in these evaluations overestimate the bias in LDL-chol of
the Friedewald approach, especially for lipemic plasma. In
plasma samples with TG >8.0 mmol/L the direct method is
preferred over the Friedewald approach. Until now, large-scale
use of the direct method is prevented by the instability of the
analyte when stored frozen [20]. Irrespective of the value of any
method in the risk estimation for coronary heart disease, the
support of a lipid reference laboratory remains necessary be­
cause of the limited value of both the Friedewald approach and
the direct LDL-method for proper phenotyping of strongly
hyperlipidemic samples. Considering the fact that the measured
plasma triglyceride concentration also gives insight into the
5. Jialal I, Hirany SV, Devaraj S, Sherwood TA. Comparison of an immunopré­
cipitation method for direct measurement of LDL-chol with beta-quantitation
6. Mariotti J, Mäki J, Mänttäri J, Järvisen O, Impeaer O. Poor applicability of
the Friedewald formula in the assessment of serum LDL cholesterol for
We thank Sigma Diagnostics for providing the reagents of the
immunoseparation kit.

References
1. Report of the National Cholesterol Education Program Expert Panel on
detection, evaluation and treatment of high blood cholesterol in adults. Arch
2. McNamara JR, Cohn JS, Wilson PWF, Schaefer EJ. Calculated values for
low-density lipoprotein cholesterol in the assessment of lipid abnormalities
4. Mariotti J, Mäki J, Mänttäri J, Järvisen J, Impeaer O. Poor applicability of
the Friedewald formula in the assessment of serum LDL cholesterol for
5. Jialal I, Hirany SV, Devaraj S, Sherwood TA. Comparison of an immunopré­
cipitation method for direct measurement of LDL-chol with beta-quantitation
6. Mariotti J, Mäki J, Mänttäri J, Järvisen J, Impeaer O. Poor applicability of
the Friedewald formula in the assessment of serum LDL cholesterol for

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A characteristic rise and fall in the concentrations of creatine kinase (CK) and its MB isoenzyme are often used as indicators of myocardial damage [1]. However, CK may be abnormally high in up to 90% of patients with hypothyroidism [2]. This increase is mostly from increased MM, but MB has also been reported to increase above reference values in hypothyroid patients without apparent myocardial damage [3]. Hypothyroid patients experience skeletal muscle signs and symptoms, including muscle cramps, stiffness, myalgias, and myocidemia [4]. However, even some without muscle complaints have increased CK, probably because of decreased clearance of CK and CK-MB [5].

Coronary atherosclerosis occurs twice as frequently in patients with hypothyroidism as in age- and sex-matched controls [6]. Because of their increased tendency to develop hypertension and hypercholesterolemia, hypothyroid patients are predisposed to coronary artery disease and subsequently to myocardial infarction. Saito et al. found that hypertension was more frequent in hypothyroid patients than in age-matched euthyroid persons [6]. Also, cholesterol-fed animals with hypothyroidism developed accelerated atherosclerosis that is reduced after thyroid hormone replacement [7]. These observations suggest that the concomitant occurrence of ischemic heart disease and hypothyroidism will be a recurring concern.

Recently, the new markers troponin T and troponin I (Tn-I) have been extensively studied because of their cardiac specificity. Their concentrations increase within 6 h of myocardial injury and remain increased for as long as 7 days. However, increased concentrations of troponin T have also been found in polymyositis/dermatomyositis [8], renal failure [9], trauma [10], and rhabdomyolysis [10], whereas no increase in Tn-I is seen in patients with rhabdomyolysis, multiple trauma, chronic muscle disease, chronic renal failure, or in marathon runners [11–15]. Given the apparent lack of published reports on Tn-I values in hypothyroidism and in view of the difficulty in interpreting CK and CK-MB, we studied the effect of hypothyroidism on Tn-I.

A case that illustrated the above points and was the impetus for this study was that of a 55-year-old white man who presented to the hospital with the complaint of intermittent left-side chest pain and progressive shortness of breath and edema for 6 months. He had a history of an anterosetal myocardial infarction in 1982 and complained of constipation and weight gain. Chest x-ray revealed cardiomegaly and congestive failure, and echocardiogram showed a pericardial effusion. Laboratory values were as follows: CK 9160 U/L (reference range 50–150 U/L for men and 40–120 U/L for women), CK-MB 21 μg/L (reference range 0–4 μg/L), and cardiac Tn-I <0.4 μg/L (reference range <0.4 μg/L). On the basis of these findings, he was ruled out for acute myocardial infarction.

Subsequently, we reviewed 52 consecutive patients presenting to Parkland Memorial Hospital in late January and early February 1996 in whom a thyrotropin (TSH) value >25 mU/L was observed (reference range 0.4–4.5 mU/L). The procedures we followed were within the standards set by the Ethics Committee of our institution. Because this was a laboratory-initiated study, we cannot report on the severity of the hypothyroidism based on clinical findings, and the findings in this study should be interpreted in that light. Most of these patients were seen in the outpatient clinic for management of their hypothyroid state. Various etiologies of hypothyroidism were observed. Two patients had atypical chest pain, but infarction was excluded. Ages ranged from 26 to 70 years (mean 46), an age group not uncommonly encountered in the evaluation of ischemic heart disease. The patients studied were 42 women and 10 men. TSH values ranged from 25.1 to 295.5 mU/L (mean 79.7). Free thyroxine (FT₄) values ranged from undetectable to 12.9 pmol/L (reference range 10.3–23.2 pmol/L). The same sample used to measure TSH and FT₄ was also used to measure CK, CK-MB, cholesterol, and cardiac Tn-I. TSH and FT₄ were measured on the Ciba Corning (Medfield, MA) ACS-180 with an immuno-