EDTA-Plasma vs Serum Differences in Cholesterol, High-Density-Lipoprotein Cholesterol, and Triglyceride as Measured by Several Methods

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To investigate EDTA-plasma/serum (P/S) differences, we collected paired samples from 25 volunteers and measured total cholesterol (TC), triglyceride (TG) and high-density-lipoprotein cholesterol (HDLCL), using the Cobas FARA, Ektachem 700, DuPont Dimension, and Baxter Paramax Analyzers. The mean (SD) P/S ratios for TC, HDLC, and TG concentrations were, respectively: 0.980 (0.0171), 1.063 (0.0704), and 0.961 (0.0363) for Paramax; 0.976 (0.0189), 1.034 (0.1091), and 0.950 (0.0557) for Dimension; 1.003 (0.0221), 1.059 (0.0304), and 0.988 (0.0179) for Ektachem; and 0.993 (0.0162), 1.063 (0.0830), and 1.013 (0.0410) for Cobas. We conclude that P/S ratios vary by analytical methods, and that HDLC ratios tend to be larger in magnitude and in the opposite direction from TC and TG. Both effects lead to significant biases in computed disease risk.

Indexing Terms: sample collection/variation, source of/intermethod comparison/heart disease/risk factors

Accurate risk assignment for coronary heart disease requires both accurate and precise blood lipid measurements. Consequently, the Lipid Standardization Panel of the National Cholesterol Education Program (NCEP) has assigned accuracy (bias) and precision targets for these analytes.2 Goals for laboratory accuracy and precision of measurements of total cholesterol (TC) were promulgated in 1988 (1) as 5% and 5%, respectively, both to be reduced to 3% by 1992. Modern automated instruments appear to have quite adequate precision to meet even the more-stringent 1992 precision goal for TC (1). Formal accuracy and precision goals for triglycerides (TG) and high-density lipoprotein cholesterol (HDLC) are not yet published. Estimating actual interlaboratory accuracy (bias) for all of these analytes is difficult, because "matrix effects" in most of the materials used in interlaboratory proficiency-testing surveys make a simple comparison of interlaboratory performance by use of lyophilized proficiency-testing fluids potentially unreliable and potentially misleading (2–4). However, for laboratories that participated in the 1989 College of American Pathologists (CAP) General Chemistry Survey, in which a pooled frozen serum proficiency-testing material was used, results indicate that the vast majority (~92%) apparently met the total error goal for TC of 8.9%, i.e., 3.0% + (1.96 × 3.0%), derived from the 1992 NCEP separate accuracy and precision targets (2).

Proficiency testing, even with proficiency-testing fluids that ideally mimic fresh human serum, can measure only a laboratory's within-laboratory analytical performance. It cannot measure pre- or postanalytical factors that may also markedly affect a patient's laboratory test result. Among the many nonanalytical factors that influence a patient's blood lipid results (5), one of the more important preanalytical factors that can introduce systematic bias in determinations of lipids and lipoproteins is specimen type, namely, serum vs EDTA-plasma. Both specimen types have been widely used in large research studies: e.g., the Coronary Primary Prevention Trial (6, 7) analyzed EDTA-plasma; the Helsinki Heart Study, serum (8). More recently, serum appears to have become the more widely used and recommended specimen (9, 10), although some investigators still consider EDTA-plasma preferable, particularly when lipoprotein and apolipoproteins are to be analyzed (10).

Because both EDTA-plasma and serum are used widely for lipid analyses, plasma/serum (P/S) differences have been the subject of several studies. An early report indicated that EDTA-plasma TC concentrations averaged ~3% lower than those in simultaneously collected serum (11), and the first NCEP Adult Treatment Panel report, which decided on clinical decision cut-points for serum TC [5.18 and 6.22 mmol/L (200 and 240 mg/dL)], recommended that EDTA-plasma TC results be multiplied by 1.03 for interpretation by NCEP guidelines (9). The observed 3% difference was attributed to a change in fluid shifts between plasma and erythrocytes in the presence of EDTA. A later study found a P/S TC difference of 4.7% and attributed the larger bias to an increase of ~50% in EDTA concentration in more-recently manufactured commercial evacuated blood-collection tubes (12).

Less has been published on EDTA-plasma vs serum biases in HDLC and TG determinations. In addition, formal accuracy and precision goals for these two analytes are yet to be published by the NCEP Laboratory Standardization Panel. However, preliminary analytical performance goals for accuracy and precision of HDLC determinations have been selected as 10% and 6%, respectively, by 1994 and 5% and 4% by 1998 (M. Kimberly, Cholesterol Reference Method Laboratory Network, Centers for Disease Control and Prevention, personal communication). In some earlier reports from the Lipid Research Clinic laboratories on the effect of
n manganese ion concentration and EDTA-plasma vs serum on HDLC results, a reasonably complete precipitation of apolipoprotein B-containing lipoprotein particles was found to require greater manganese concentrations for EDTA-plasma samples than for serum samples (13, 14). Nevertheless, the P/S bias for HDLC analyzed by the Lipid Research Clinics chemical-extraction method on the AutoAnalyzer II was reportedly <1% (15). The average P/S ratio for TG has been reported to be between 0.97 (11) and 0.95 (15), close to the P/S ratios reported for TC.

The P/S biases for the major lipid and lipoprotein fractions that are used for prediction of atherosclerotic disease risk and for therapeutic decisions appear to be as great as the proposed maximum analytical bias under NCEP Laboratory Standardization Panel guidelines. Thus, we decided to investigate more systematically the P/S biases for a centrifugal analyzer method we planned to use for several upcoming NIH-funded multicenter research studies for which specimens were to be assayed in our laboratory. At the same time, we decided to investigate some of the other commonly used clinical methods.

Materials and Methods

Subjects and Specimens

The study population consisted of 25 apparently healthy individuals (6 men and 19 women; ages 25–50 years, average 39.5 years). The study was approved by our institutional review board. After an overnight fast, 15 mL of venous blood was collected with a butterfly needle and a 30-mL plastic syringe. The blood was mixed in the syringe for few seconds and then aliquoted into evacuated 7.0-mL (blood volume) blood-collection tubes (Vacutainer Systems; Becton-Dickinson, Rutherford, NJ) that contained either no anticoagulant (prod. no. 6431) or disodium EDTA designed to give a final concentration in blood of 4.46 mmol/L (prod. no. 6452, lot no. 4C020). This plastic syringe technique was used to eliminate any temporal effects on lipoprotein concentration from tourniquet application. The EDTA-anticoagulated samples were cooled to 4°C without delay. Samples collected without anticoagulant were allowed to clot at room temperature for 45 ± 10 min. Cellular components were removed from both sample types by centrifugation at 1500g at 60 ± 15 min after venipuncture. Separated serum and plasma specimens were then stored at 4°C for ≤48 h before analysis.

Lipid Analyses

TC, HDLC, and TG were measured in each serum specimen and in each plasma specimen in duplicate. To minimize the impact of run-to-run variability, we always assayed the matched EDTA-plasma and serum pairs from a given volunteer in the same analytical batch. Analytical methods used included:

1) Cobas FARA. Cholesterol oxidase-based reagent (prod. no. 236691) and glycerol oxidase-based TG reagent (prod. no. 701912) were supplied by Boehringer Mannheim (Indianapolis, IN). We used the HDLC method of Warnick et al. (16), combining 0.50 mL of serum or plasma with 50 μL of a solution containing 0.50 mol/L magnesium chloride and 10 g/L dextran sulfate (average molecular mass 50 kDa; Genzyme, Cambridge, MA; prod. no. 70-5801-00), vortex-mixing ~10 s, incubating for 10 min at room temperature, and centrifuging (1500g, 10 min) before analyzing cholesterol in the supernate.

2) Ektachem 700. The reagent slides for cholesterol (prod. no. 168-8290), TG (prod. no. 184-8088), and HDLC precipitation reagent (prod. no. 146-32356) were supplied by Kodak (Rochester, NY) and used as directed by the manufacturer. In the Kodak HDLC method 0.50 μL of serum or plasma is combined with a premeasured amount of HDLC reagent to yield 0.91 g/L dextran sulfate (50 kDa) and 45 mmol/L magnesium chloride in the final precipitation mixture. After vortex-mixing for ~10 s, incubating 10 min, and centrifuging (1500g, 10 min), the supernate cholesterol is analyzed with the same total cholesterol slides, calibrated specifically for HDLC analysis.

3) DuPont Dimension. The TC (prod. no. DF-27), TG (prod. no. DF-69), and HDLC (prod. no. DF-47) reagents were supplied by DuPont (Wilmington, DE) and used as directed by the manufacturer. The DuPont HDLC method combines 0.250 mL of serum or plasma with 50 μL of 6.0 mmol/L phosphotungstic acid; after vortex-mixing (~5 s), incubation (room temperature, 6 min), and centrifugation (1500g, 20 min), the cholesterol in the supernate is assayed.

4) Paramax. The reagent kits for all three analytes were supplied by Baxter Travenol (Chicago, IL) and used as directed by the manufacturer. The Baxter HDLC method combines 0.50 mL of serum or plasma with 100 μL of 6.59 mmol/L phosphotungstic acid, vortex-mixes (~10 s), incubates (10 min), and centrifuges (1500g, 10 min), and then analyzes cholesterol in the supernate.

5) TC was quantified in our laboratory by the modification of the original Abell–Kendall method that is currently used at the Centers for Disease Control and Prevention (16). During this study our laboratory’s primary methods for TC, HDLC, and TG analysis for multicenter research studies were as listed above for the Cobas FARA. These methods were continually standardized via the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention’s Lipid Standardization Program.

Computations were performed on a Macintosh IIx computer with Excel software (Microsoft, Redmond, WA) for computation of the means of duplicates and calculated low-density lipoprotein cholesterol (LDLC) concentrations with the Friedewald et al. equation (17). Statistical analyses were also performed on the Macintosh IIx with Statview II (BrainPower, Calabasas, CA). The mean and SD for P/S ratio for calculated LDLC for a given method were derived by computing a calculated LDLC for each volunteer’s EDTA-plasma, computing a calculated LDLC for that same volunteer’s matched se-
rum, dividing the EDTA-plasma calculated LDLC result by its matched serum result, and finally computing the mean and SD from the 25 individual LDLC P/S ratios.

**Results and Discussion**

Table 1 shows the mean (SD) concentrations of the various lipids and lipoproteins measured in serum and in EDTA-plasma as well as the mean (SD) P/S ratios for the 25 volunteers for each of the analytical methods used. The lipid and lipoprotein concentrations were what would be expected for a healthy, reasonably young population.

We found several results in Table 1 quite interesting. First, the mean P/S ratio for TC is statistically different across analytical methods. The original difference between EDTA-plasma and serum TC was reported to be ~2–3% when TC was analyzed by chemical extraction methods (11, 15). Cloey et al. (12), using a cholesterol oxidase-based method for TC, later reported a TC P/S difference of 4.7%. They attributed their observed increase in the P/S bias to an increase in the amount of disodium EDTA in the collected blood from ~3.0 mmol/L in older EDTA blood-collection tubes to 4.5 mmol/L in more modern commercially available EDTA-containing tubes, a change that occurred in the mid-1980s. However, our findings indicate that the P/S ratio for TC for some analytical methods is statistically different (two-tailed, unpaired Student’s t-test) from that for the Abell–Kendall method (0.984): Cobas ratio 0.993 (P = 0.02); Ektachem ratio, 1.003 (P = 0.0002); Paramax ratio, 0.980 (P = 0.40); Dimension ratio, 0.976 (P = 0.08). We consider very unlikely that the Abell–Kendall TC reference method with its extraction step would be affected by changing the sample matrix from EDTA-plasma to serum. Thus, we believe the Abell–Kendall P/S ratio should very closely approximate the true P/S ratio for TC concentration. Because some of the other analytical methods yield P/S TC ratios that are statistically very significantly different from the Abell–Kendall TC P/S ratio, we suggest that part of the observed change in P/S bias reported by Cloey et al. in 1990 is a result of the change in their analytical method for TC—from a chemical extraction method to an enzymatic method—rather than the difference being entirely due to an increased EDTA concentration in blood-collection tubes.

Our observed P/S ratios for TG with three of the four methods we assessed tend to be in the same direction and roughly the same order of magnitude as previously reported (11, 15). For three of the TG methods, the TG P/S ratios are statistically very significantly different from the P/S ratio for TC (0.984) by Abell–Kendall analysis: Paramax ratio, 0.961 (P = 0.005); Dimension ratio, 0.950 (P = 0.005); Ektachem ratio, 0.988 (P = 0.32); and Cobas ratio, 1.013 (P = 0.001). Although it is probable that the true TG concentration in EDTA-plasma vs serum may actually differ, the fact that the P/S ratios for TG differ in a statistically significant fashion from the Abell–Kendall TC P/S ratio, and from each other, can be explained, we believe, only if the clinical enzymatic TG methods recover TG differently from EDTA-plasma than from serum. Because there are no widely available or accepted reference or definitive methods for TG, distinguishing unequivocally the magnitude of analytical recovery biases vs true systematic differences in TG concentration in EDTA-plasma vs serum is not possible.

Our original primary purpose for beginning this study was to investigate carefully the EDTA-plasma vs serum difference for HDLC methods. It is interesting to note

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**Table 1.** Measured serum concentration, EDTA-plasma concentration, and P/S concentration ratios for total cholesterol, HDL-cholesterol, triglycerides, and calculated LDL-cholesterol determined by various analytical methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Specimen or ratio</th>
<th>Cholesterol (SD), mmol/L</th>
<th>HDLC (SD), mmol/L</th>
<th>Triglycerides (SD), mmol/L</th>
<th>Calculated LDL (SD), mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paramax</td>
<td>Serum</td>
<td>5.02 (1.04)</td>
<td>1.37 (0.42)</td>
<td>1.50 (1.29)</td>
<td>2.96 (1.08)</td>
</tr>
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<td></td>
<td>EDTA-plasma</td>
<td>4.93 (1.04)</td>
<td>1.44 (0.40)</td>
<td>1.45 (1.26)</td>
<td>2.82 (1.11)</td>
</tr>
<tr>
<td></td>
<td>P/S ratio</td>
<td>0.980 (0.0171)</td>
<td>1.063 (0.0704)</td>
<td>0.950 (0.0363)</td>
<td>0.94 (0.0649)</td>
</tr>
<tr>
<td>Dimension</td>
<td>Serum</td>
<td>5.29 (1.08)</td>
<td>1.43 (0.40)</td>
<td>1.39 (1.34)</td>
<td>3.29 (1.18)</td>
</tr>
<tr>
<td></td>
<td>EDTA-plasma</td>
<td>5.17 (1.07)</td>
<td>1.47 (0.40)</td>
<td>1.31 (1.25)</td>
<td>3.10 (1.18)</td>
</tr>
<tr>
<td></td>
<td>P/S ratio</td>
<td>0.976 (0.0189)</td>
<td>1.034 (0.1910)</td>
<td>0.950 (0.0557)</td>
<td>0.94 (0.0850)</td>
</tr>
<tr>
<td>Ektachem</td>
<td>Serum</td>
<td>5.13 (1.00)</td>
<td>1.39 (0.38)</td>
<td>1.53 (1.39)</td>
<td>3.04 (1.08)</td>
</tr>
<tr>
<td></td>
<td>EDTA-plasma</td>
<td>5.15 (1.03)</td>
<td>1.47 (0.39)</td>
<td>1.50 (1.32)</td>
<td>3.00 (1.11)</td>
</tr>
<tr>
<td></td>
<td>P/S ratio</td>
<td>1.003 (0.0221)</td>
<td>1.059 (0.0304)</td>
<td>0.988 (0.0179)</td>
<td>0.979 (0.0399)</td>
</tr>
<tr>
<td>Cobas</td>
<td>Serum</td>
<td>5.05 (1.04)</td>
<td>1.33 (0.39)</td>
<td>1.44 (1.26)</td>
<td>3.06 (1.09)</td>
</tr>
<tr>
<td></td>
<td>EDTA-plasma</td>
<td>5.01 (1.01)</td>
<td>1.40 (0.39)</td>
<td>1.43 (1.22)</td>
<td>2.95 (1.07)</td>
</tr>
<tr>
<td></td>
<td>P/S ratio</td>
<td>0.993 (0.0162)</td>
<td>1.063 (0.0830)</td>
<td>1.013 (0.0410)</td>
<td>0.961 (0.0308)</td>
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<tr>
<td>Abell–Kendall</td>
<td>Serum</td>
<td>5.04 (1.05)</td>
<td>1.46 (1.04)</td>
<td>1.46 (0.39)</td>
<td>2.85 (1.07)</td>
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<tr>
<td></td>
<td>EDTA-plasma</td>
<td>4.96 (1.04)</td>
<td>0.984 (0.0113)</td>
<td>1.063 (0.0830)</td>
<td>1.013 (0.0410)</td>
</tr>
</tbody>
</table>

* Cholesterol mmol/L concentration can be converted to mg/dL by multiplying by 38.6 and triglyceride (as triolein) mmol/L concentration to mg/dL by multiplying by 88.5. n = 25 each.

b–d Significantly different from serum mean by the same analytical method by paired, two-tailed Student’s t-test: b \( P < 0.001 \); c \( 0.001 < P < 0.01 \); d \( 0.01 < P < 0.05 \); others are not significant \( P > 0.05 \).
that the P/S bias for HDLC is in the opposite direction from that for TC. Furthermore, the P/S bias for HDLC in some methods is as large or larger than the proposed maximum allowable analytical bias for HDLC. Substantial and analytical method-specific P/S ratios for HDLC are not totally unexpected. The exact manganese concentration is known to be an important variable for the manganese-heparin HDLC methods used by the Lipid Research Clinics; and, as expected, increasing the manganese concentration leads to lower HDLC results (14). In fact, the 46 mmol/L manganese chloride concentration in the mixture used for the Coronary Primary Prevention Trial to precipitate LDLC and very-low-density lipoprotein cholesterol (VLDLC) (i.e., apolipoprotein B-containing lipoproteins) was probably somewhat low; it has since been suggested (18) that 92 mmol/L manganese would have been more appropriate for EDTA-plasma, which was the specimen type used in that landmark drug study. The problem created by the presence of EDTA is that chelated metal ions do not participate effectively in the precipitation of LDLC and VLDLC by metal ion-containing HDLC reagents (e.g., manganese-heparin and magnesium-dextran). The differences in VLDLC and LDLC precipitation efficiency between EDTA-plasma and serum seem to have been partially forgotten in the late 1980s and '90s. Many manufacturers' package inserts (e.g., Baxter Paramax, DuPont Dimension) and clinical laboratories list both EDTA-plasma and serum as "acceptable" specimen types for HDLC quantification without discussing the preanalytical biases introduced by the type of collection tube. Interestingly, Kodak's package insert indicates that HDLC in EDTA-plasma is 0.06–0.08 mmol/L (2.2–3.2 mg/dL) higher than in simultaneously collected serum and references a 1985 study on a magnesium–50-kDa dextran sulfate method performed on the Ektachem 400 (19). The P/S bias in that study is amazingly close to what we have found in 1994 with the Ektachem 700. Also noteworthy is the fact that the P/S biases for all of our HDLC methods significantly exceed the early report that EDTA-plasma and serum are "virtually identical" with the Lipid Research Clinic's manganese–heparin precipitation method and the AutoAnalyzer II TC method (15). Because the EDTA-plasma HDLC result tends to be biased high compared with serum while its TC tends to be biased low, the LDLC concentration computed by using the Friedewald equation (17) is quantitatively biased even lower. This situation in turn further increases the low bias of the EDTA-plasma LDLC/HDLC ratios, which are widely used as the starting point for calculations of coronary disease risk estimations. For example, the LDLC/HDLC ratio for an individual's EDTA-plasma averages only 88.5%, 91.3%, 92.4%, or 90.4% of the ratio that would have been determined had serum been collected and analyzed by the Paramax, Dimension, Ektachem, or Cobas, respectively. Also, one should remember that partial filling of EDTA-containing blood-collection tubes would be expected to increase quite markedly the magnitude of the observed P/S biases in measured and calculated lipids and lipoproteins, and in the coronary disease risk computed from them.

In conclusion, if one considers the laboratory standardization efforts being spent to reduce analytical bias to <3% for TC and <5% for HDLC, it is clear that laboratories and clinicians should be cognizant of the comparably large preanalytical biases introduced by changing between serum and EDTA-plasma samples. Furthermore, manufacturers of diagnostic reagents and instruments for TC, TG, and HDLC—and the clinical laboratories that use them—should provide information of the exact quantitative biases introduced by use of EDTA-plasma, rather than serum, if they consider EDTA-plasma acceptable for these analyses.

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References
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