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A Cellulose Acetate Electrophoretic Procedure Evaluated for Quantitation of High-Density Lipoprotein Cholesterol
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We evaluated the performance of a commercially available cellulose acetate electrophoretic method for quantititating high-density lipoprotein cholesterol (I) in serum by comparing it to a method involving precipitation with dextran sulfate-500/Mg2+. In both methods, enzymic reagents are used for cholesterol measurement. For electrophoretic measurement of I the mean intramembrane CV was 4.1% (at 220 to 360 mg/L) and the intermembrane CV ranged from 12.2 to 21.0% (at 220 to 880 mg/L). Interassay precision was significantly better for the precipitation method (CV = 3.9% at 390 mg/L). The electrophoretic procedure demonstrated significant measurement bias, both at high and low I concentrations. However, low-density lipoprotein cholesterol, measured electrophoretically, correlated well with its calculated concentrations obtained by the precipitation method. Measurements of I by this electrophoretic procedure did not achieve the accuracy and reproducibility that have been demonstrated for precipitation methods and that are necessary for reliable clinical interpretation of results for I.

The concentration of high-density lipoprotein cholesterol (HDLC) in serum is an independent negative risk factor for the development of coronary artery disease and has become an important component of the lipid profile (1). As a result, HDLC assays have received increased attention and evaluation. Measurement of HDLC involves two steps: high-density lipoprotein (HDL) is separated from other serum lipoproteins and cholesterol is measured in the isolated HDL fraction.

Burstein (2) described a precipitation technique involving divalent cations and polyvalent anions for effectively separating other serum lipoproteins from HDL. This method, with modifications, has largely replaced more cumbersome ultracentrifugation methods for HDLC isolation and was the method used in the Framingham heart epidemiologic study that demonstrated the inverse relation between HDLC and coronary artery disease (3).

Several important factors must be considered in quantitating HDLC. Its concentration in serum is generally fourfold lower than the value for total serum cholesterol, so methods for measuring HDLC must therefore have good sensitivity and linearity. In addition, unlike total cholesterol measurements, major changes in interpretation are associated with a narrow range of HDLC values, so that analytical precision is critical. Finally, individual laboratories cannot independently determine interpretive criteria for HDLC but must rely on previously reported longitudinal studies. Therefore, good method comparison with reference laboratories is essential.

Several types of precipitation methods coupled with enzymic and nonenzymic cholesterol quantitation have been reported; nearly all show the necessary accuracy and precision needed for meaningful interpretation (4). Recently, a different electrophoretic approach has become available for HDLC separation and quantitation (5). After serum electrophoresis, an enzymic cholesterol reagent is applied to the membrane and HDLC is measured with a scanning densitometer as a percentage of the total cholesterol. This approach has several advantages, including small sample size, direct total cholesterol/HDLC results, and compatibility with other clinical laboratory electrophoretic techniques. However, HDLC quantitation by electrophoresis is a major departure from precipitation methods, and results should be critically compared to those obtained by other generally used methods before this technique is recommended for routine use. We describe here our comparison of two methods for measuring HDLC: a well-standardized precipitation method involving dextran sulfate-500/Mg2+ with enzymic cholesterol reagents.
and an electrophoretic method involving commercially available cellulose acetate membranes and enzymic cholesterol reagents.

Material and Methods

**HDLC and LDLC by electrophoresis:** Serum HDLC and LDL c were separated and quantitated on cellulose acetate membranes by the electrophoretic HDL-cholesterol method available from Helena Laboratories, Beaumont, TX 77704. We performed the assays using the procedure outlined by Helena. Membranes were scanned with the Cliniscan densitometer (Helena Laboratories). This method is described in detail by Cobb et al. (5).

**HDLC and LDL C by precipitation:** HDLC was quantitated by a modification (6) of the method of Kostner (7), in which dextran sulfate-500/Mg2+ is the precipitating agent, and cholesterol is quantitated with enzymic reagents. LDL C was calculated by the formula LDL C = TC - (HDLC+ TG/5) (8). None of the samples had the creamy layer indicative of chylomicrons.

Intramembrane precision was determined by using sera from four patients with low concentrations of HDLC (≤360 mg/L). The samples were paired and analyzed on eight membranes during four days. Intramembrane precision was determined by using sera from five patients, four of whom had low HDLC concentrations (≤410 mg/L) and one of whom had an HDLC concentration of 880 mg/L. Each sample was analyzed on 15 different membranes within four days of collection.

We also evaluated interassay precision, using Ortho normal lyophilized control serum, unassayed (Ortho Diagnostics, Raritan, NJ 08869). This control serum was diluted twofold in normal saline and designated as pool I. Undiluted Ortho control serum was designated as pool II. We did 57 precipitation and 16 electrophoretic HDLC assays on pool I, and 59 precipitation and 20 electrophoretic assays on pool II.

Sera from 99 consecutive patients were analyzed for HDLC and LDL C by both electrophoresis and precipitation. The data were analyzed statistically with the aid of a 9845T computer (Hewlett-Packard, Fort Collins, CO 80525).

**Results**

**Precision.** We evaluated intramembrane precision by measuring the HDLC concentration on four sets of paired patient samples, using eight membranes (four pairs per membrane) (Table 1). We further evaluated intramembrane precision by measuring the HDLC concentration on five additional patient samples, using 15 membranes (Table 2). Using lyophilized control sera at two different concentrations, we compared the interassay precision of the electrophoretic method with that for the precipitation method (Table 3). The former showed greater measurement variability than the latter (pool I, F-ratio = 3.36, p < 0.001; pool II, F-ratio = 2.88, p < 0.001).

**Accuracy.** We used 99 serum samples to compare the electrophoretic method with the precipitation method. The TC concentrations of these samples ranged from 910 to 3520 mg/L (mean, 2150 mg/L). The TG concentrations ranged from 420 to 3220 mg/L (mean, 1338 mg/L). The mean HDLC concentration was 445 (SD 131) mg/L by the precipitation method and 476 (SD 209) mg/L by the electrophoretic method. A wider range of HDLC values was obtained by electrophoresis (110–1020 mg/L) than by precipitation (240–810 mg/L). LDL C concentrations as measured by electrophoresis ranged from 660 to 2790 mg/L, with a mean of 1508 (SD 459) mg/L. Calculated LDL C concentrations ranged from 460 to 2710 mg/L with a mean of 1501 (SD 442) mg/L.

Figure 1 summarizes the comparison between HDLC results obtained by electrophoresis and by precipitation. The slope of the linear regression line is 1.42 (95% confidence interval, 1.27 to 1.56) and the y-intercept is −150 mg/L (95% confidence interval, −220 to −90 mg/L). There was a significant difference between means by the paired t-test (t = −2.85, p < 0.01). Method bias was related to the concentration of serum HDLC. At low HDLC concentrations electrophoretic values were lower than precipitation values, and vice versa.

Figure 1 also shows a between-method correlation for LDL C measurement. The slope of the linear regression line is 0.95 (95% confidence interval, 0.87 to 1.04) and the y-intercept is 80 mg/L (95% confidence interval, −50 to 210 mg/L). There was no difference between electrophoretic and calculated LDL C values by the paired t-test (t = −0.38, p > 0.05).

**Discussion**

Electrophoretic techniques are widely used to separate serum lipoproteins, which are then detected by staining with various lipophilic dyes, but lipoprotein electrophoresis is semiquantitative and may not be as useful in providing an assessment of a patient's lipid status as measurement of total cholesterol and triglyceride (9). Quantitation of cholesterol in lipoprotein fractions separated by ultracentrifugation or precipitation methods provides more sensitive and reliable diagnostic and prognostic information (10). Recently, serum electrophoresis coupled with lipoprotein cholesterol quantitation by enzymic staining was developed as an alternative to precipitation and ultracentrifugation methods (5). We compared the precision and accuracy of this electrophoretic method with a standard dextran sulfate-500/Mg2+ precipitation procedure.

The reliability of the precipitation method we used for this

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**Table 1. Summary of Repeated HDLC Measurements by Electrophoresis**

<table>
<thead>
<tr>
<th>Mean HDLC, mg/L</th>
<th>Intramembrane CV, %</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>220</td>
<td>3.4</td>
</tr>
<tr>
<td>260</td>
<td>4.8</td>
</tr>
<tr>
<td>290</td>
<td>3.6</td>
</tr>
<tr>
<td>360</td>
<td>4.3</td>
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</tbody>
</table>

* Four patients’ sera; n = 8 for each.

**Table 2. Intramembrane Precision of HDLC Measurement by Electrophoresis**

<table>
<thead>
<tr>
<th>Mean HDLC, mg/L</th>
<th>Intramembrane CV, %</th>
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<tbody>
<tr>
<td>HDLC</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>18.7</td>
</tr>
<tr>
<td>310</td>
<td>21.0</td>
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<td>350</td>
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<tr>
<td>410</td>
<td>16.4</td>
</tr>
<tr>
<td>880</td>
<td>14.3</td>
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</table>

* Five patients’ sera; n = 15 for each.

**Table 3. Interassay Precision of HDLC Measurements for Two Lyophilized Control Pools**

<table>
<thead>
<tr>
<th>Mean HDLC, mg/L</th>
<th>Interassay CV, %</th>
<th>Mean HDLC, mg/L</th>
<th>Interassay CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>130</td>
<td>16.7</td>
<td>57</td>
</tr>
<tr>
<td>20</td>
<td>320</td>
<td>8.0</td>
<td>59</td>
</tr>
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</table>

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study is well documented. Warnick et al. (4) compared six different precipitation methods and demonstrated that the dextran sulfate-500/Mg\(^{2+}\) method had precision equal to or better than all other precipitation procedures evaluated. Finley et al. (6) demonstrated excellent agreement between results by the dextran sulfate-500/Mg\(^{2+}\) and Cooperative Lipoprotein Phenotyping Study methods. The electrophoretic method we used is commercially available and cellulose acetate membranes are used.

The overall correlation between the electrophoretic and precipitation procedures demonstrated a significant nonlinearity and bias that was a function of the HDLc concentration. At low HDLc concentrations the electrophoretic method gave consistently lower values than the precipitation method. At high HDLc concentrations electrophoretic values were consistently higher. The linear regression line had a slope and y-intercept significantly different from 1 and 0, respectively, at the 95% confidence level. In addition, the difference between method results was significant by the paired t-test (t = 2.85 p < 0.01). These results are in agreement with Stein et al. (11), who evaluated an agarose gel electrophoretic method and found it gave consistently lower values at low HDLc concentrations when compared to a heparin-Mn\(^{2+}\) precipitation method. In a similar evaluation Bullock et al. (12) compared HDLc values by agarose gel electrophoresis and phosphotungstate-Mg\(^{2+}\) precipitation and demonstrated a linear regression line (y = 1.51x - 216) similar to what we observed (y = 1.42x - 154). Conlon et al. (13) did not demonstrate any difference between agarose gel electrophoresis and precipitation methods, although they did not separately evaluate a subset of their low HDLc data as Stein did. Cobb et al. (5) demonstrated good correlation on comparing HDLc results from cellulose acetate membranes and expected HDLc values for different age groups. However, method comparison cannot be evaluated from this study since paired samples were not analyzed. Finally, Baillie et al. (14) compared both cellulose acetate and agarose gel electrophoretic methods to a phosphotungstate-Mg\(^{2+}\) precipitation method. They demonstrated significant differences (by Student's t-test) in HDLc values for agarose gel but not cellulose acetate membranes. Baillie et al., however, do not report the range of HDLc values tested, although they comment that bias was seen on samples with high and low total cholesterol levels. The above observations suggest that use of previously published interpretive criteria would be unreliable if HDLc results were obtained by electrophoretic assays, particularly at low HDLc concentrations.

We observed better intramembrane precision than has previously been reported for other electrophoretic procedures. The average intramembrane CV at HDLc concentrations of 220 to 360 mg/L was 4.1%. Conlon et al. (13) reported a CV of 4.9% for electrophoresis on agarose gel, although the mean HDLc concentration was 574 mg/L. Cobb and Sanders, using cellulose acetate membranes (5), reported an intramembrane precision of 12.1% at a concentration of 389 g/L. Our 3-fold improvement in intramembrane precision may be due to the higher-quality membranes and (or) better instrumentation for densitometric scanning (Cliniscan) that are now generally available.

We observed an intermembrane precision (CV) for patient's samples and lyophilized controls that ranged from 12.2 to 21.0%. Conlon et al. (13), using agarose gel membranes, reported an intermembrane precision of 5.1% for a 574 mg/L concentration. However, the high concentration of HDLc chosen to evaluate intermembrane precision in their study may be misleading, because in a similar evaluation Stein et al. (11), using agarose gel electrophoresis, reported interassay CVs of 3.5, 4.3, 12.6, and 20.0% at HDLc concentration of 657, 523, 380, and 160 mg/L, respectively. This agrees with the results that we and Cobb and Sanders (5) obtained with cellulose acetate membranes. Baillie et al. (14) observed a somewhat better intermembrane CV of 8.4% for a 350 mg/L concentration with agarose gel.

The precipitation procedure we used has significantly better interassay precision than have electrophoretic methods. This agrees with other evaluations of precipitation methods (4, 5, 15). These results suggest that electrophoretic methods may be less desirable, because good reproducibility is needed for reliable interpretation of HDLc results, particularly at low, "high-risk" HDLc concentrations. It has been suggested that a CV lower than 8% (at the level of 400 mg/L) (16) "or technical error of less than 50 mg/L" (1) or better should be obtained for HDLc assays. Such precision is consistently achievable with various precipitation methods but not with electrophoretic methods.

LDLc measurement by electrophoresis correlates well with calculated LDLc values. The LDLc results show good linearity, and results by both methods are not significantly different by the paired t-test. The reason for the difference between the LDLc and HDLc method comparison results is not
clear. Perhaps LDL measurements correlate better because of the higher concentration of cholesterol being assayed. However, at higher HDLC concentrations we still observed method bias. Finally, the nonlinearity we demonstrated between the precipitation and electrophoretic procedure may be a function of the cellulose acetate membranes or possibly of the enzymic reagent–membrane interaction.

We conclude that, currently, precipitation methods are to be preferred over electrophoretic procedures for measuring serum HDLC because of their better reproducibility and sensitivity.

References