Plasma Lipoprotein Analysis: Relative Precision of Total Cholesterol and Lipoprotein-Cholesterol Measurements in 12 Lipid-Research-Clinics Laboratories

Paul S. Bachorik, Bernard Most, Kenneth Lippel, John J. Albers, and Peter D. S. Wood

The 12 laboratories of the North American Lipid Research Clinics performed lipid and lipoprotein analyses on aliquots of 53 freshly prepared plasma samples during four years. The samples, from normal, mildly hyperlipidemic, and treated hyperlipidemic subjects, were analyzed for total cholesterol, triglycerides, and cholesterol in very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The data were used to estimate the overall variability of lipoprotein-cholesterol measurements as compared with that of total cholesterol measurements in the 12 laboratories. The overall CVs for total cholesterol measurements were 2.5 and 2.1% for analyses performed with the AutoAnalyzer-I and -II, respectively. The corresponding CVs for triglyceride analyses were 5.0 and 5.7%. The results agreed well with those reported previously. Variabilities in VLDL-, LDL-, and HDL-cholesterol measurements were 2.5–3.5 times those for total cholesterol. Much of this increase was accounted for by the random component of variability and was probably caused by manipulations necessary to prepare the lipoprotein fractions for analysis. A qualitative assessment of the presence or absence of β-VLDL ("floating beta" lipoproteins), "sinking prebeta" lipoproteins, chylomicrons, and plasma turbidity, was made in 27 samples. The number of samples for which unanimity of interpretation was attained was used as a measure of comparability. The proportion of samples for which complete agreement was reached for the four components was: chylomicrons, 78%; β-VLDL, 74%; "sinking prebeta" lipoproteins, 56%; plasma turbidity, 37%. The proportion of samples for which at least three quarters of the laboratories agreed was: chylomicrons, 89%; β-VLDL, 93%; "sinking prebeta" lipoproteins, 70%; plasma turbidity, 85%.

During the past several years the participants in the Lipid Research Clinics (LRC) Program have used a combination ultracentrifugation/polyanion-precipitation method (1) to determine plasma lipoprotein concentrations in a large number of human subjects (2). Lipoprotein concentrations were determined in terms of their cholesterol content, and the procedure required the measurement of plasma cholesterol, triglycerides, and cholesterol in several plasma fractions.

The accuracy, precision, and inter-laboratory comparability achieved by the LRC laboratories for measurement of total cholesterol and triglycerides have been described, based on the analysis of both frozen quality-control pools used for day-to-day bench control (3, 4) and similar pools used for the external surveillance of laboratory performance (5, 6).

It would be desirable to have similar pools that could be used for the control of lipoprotein-cholesterol analyses. The plasma lipoproteins, however, are not stable for long periods in solution, and in general will not withstand freezing or lyophilization. This instability precluded the use of pooled serum or plasma for quality control of lipoprotein analyses in the usual way. Instead, the lipoprotein-cholesterol concentration of aliquots of freshly drawn, unfrozen plasma samples were measured by each LRC laboratory. The measurements allowed estimates of the overall precision achieved by the laboratories for the analysis of cholesterol contained in VLDL, in LDL, and in HDL. They also allowed estimates of the extent to which isolation of the various lipoprotein fractions reduced the precision of the lipoprotein-cholesterol measurements as compared with the precision achieved in measuring total plasma cholesterol.

The presence of chylomicrons, plasma turbidity, β-VLDL ("floating beta" lipoproteins characteristic of type III hyperlipoproteinemia), and "sinking prebeta" lipoproteins [Lp(a), a minor lipoprotein class with the electrophoretic mobility of α2-globulins (7)] were assessed qualitatively. The agreement among laboratories for the assessment of these variables is described.

The data reported in the present communication were collected during the conduct of the Lipid Research Clinics Prevalence Studies, from August 1972 to December 1976.

Materials and Methods

Samples

Approximately once every three months a set of three plasma samples was prepared at the Clinical Center of the National Institutes of Health, Bethesda, MD. Blood (500 mL) was withdrawn from each of three donors and collected into 600-mL blood-collection bags containing 50 mL of a 15 g/L solution of disodium EDTA (Fenwal transfer pack, 500 mL, with needle adapter; Fenwal Co., Morton Grove, IL). Plasma was collected and divided into 15-mL aliquots, and one aliquot from each subject was shipped unfrozen to each LRC laboratory. The samples were maintained at 2–4 °C during shipment.

Lipoprotein Analyses

At each LRC laboratory, the plasma was fractionated by a combination of ultracentrifugation and polyanion precipitation procedures and the cholesterol content of the fractions was determined. The methods used have been described in detail (1), are summarized in Figure 1, and can be summarized as follows. Plasma total cholesterol and triglyceride concentra-
tions were determined. Plasma (5.0 mL) was centrifuged, without density adjustment, at 105,000 × g for 18 h at 10 °C. The supernatant fraction contained lipoproteins of d <1.006 kg/L, which included VLDL and, when present, chylomicrons and β-VLDL. This fraction was removed with the aid of a tube slicer (Nuclear Supply and Service Co., Washington, DC) and stored for electrophoretic analysis. The infranatant fraction (d >1.006 kg/L), containing LDL and HDL, was reconstituted to 5.0 mL with 0.15 mol/L NaCl and its cholesterol concentration was determined.

A separate 3.0-mL aliquot of plasma was treated with heparin sulfate and MnCl₂ as described previously (4, 9) to precipitate apoB-containing lipoproteins [VLDL, LDL, Lp(a)], and HDL-cholesterol was determined by analysis of the clear supernatant fraction. VLDL- and LDL-cholesterol concentrations were calculated as follows:

\[
\text{[VLDL-cholesterol]} = \left[ \text{[total cholesterol]} - \text{[d >1.006 kg/L fraction-cholesterol]} \right] - \text{[HDL-cholesterol]}
\]

\[
\text{[LDL-cholesterol]} = \left[ \text{[d >1.006 kg/L fraction-cholesterol]} \right] - \text{[HDL-cholesterol]}
\]

Cholesterol and Triglyceride Analyses

Cholesterol and triglyceride analyses were performed on the AutoAnalyzer (AAI) or AutoAnalyzer II (AAII) (Technicon Instruments, Tarrytown, NY) as described previously (1, 3-6). Triglycerides were determined according to the procedure of Kessler and Lederer (8). The cholesterol measurements on the AAI-11 were based on the Liebermann–Burchard reaction and incorporated a serum calibration procedure (1, 9, 10) to convert observed values to values closely approximating those obtainable with the reference method of Abell et al. (11). Cholesterol was measured with the AAI by use of the FeCl₃-H₂SO₄ reaction and did not require correction of the observed values (12). All samples were analyzed in routine analytical runs, and each sample or fraction was analyzed once in each laboratory.

The cholesterol and triglyceride analyses in all participating laboratories were standardized according to criteria developed specifically for the LRC Program (13). All laboratories remained standardized throughout the course of the study.

Qualitative Determinations

Plasma (2.0 mL) was stored in a 10 × 75 mm test tube for 16 h at 4 °C. Chylomicrons, when present, accumulated as a floating cream layer, and were recorded as either “present” or “absent.” The appearance of the infranatant fraction was recorded as “clear,” “slightly turbid,” or “turbid.”

The presence of β-VLDL and “sinking prebeta” lipoproteins were determined qualitatively by subjecting the two ultracentrifugal fractions (d <1.006 kg/L, d >1.006 kg/L) to electrophoresis on paper or agarose gel (1). β-VLDL appeared as a band with β-mobility in the lipoprotein fraction of d <1.006 kg/L; sinking prebeta lipoproteins were visible as a band with pre-β mobility in the lipoprotein fraction of d >1.006 kg/L. The positions of the lipoprotein bands were identified by comparison with the electrophoretic pattern of the unfractionated plasma, i.e., each sample served as its own control. The two lipoprotein classes were recorded as either “present” or “absent.”

Experimental Design

The LRC laboratories analyzed 53 samples during August 1972-August 1976. Analyses of samples 1–26 were done in five LRC laboratories on AAI instruments from August 1972–August 1974. Samples 15–53 were analyzed with AAII instruments by 11 to 12 laboratories between January 1974–December 1976.

Precision Calculations

Each laboratory analyzed each sample once. The standard deviation for each sample was therefore calculated on the basis of five analyses for AAI measurements and 11–12 analyses for the AAII measurements and reflects combined within- and between-laboratory variation. An estimate of the precision of each variable was made by calculating the ordinary least-squares regression of standard deviation as a function of the mean concentration measured. The coefficients of variation [CV = (SD/mean concn) × 100] were calculated from the regression lines. In the present data set, CVs were not constant because the intercepts of the regression lines were not zero. This reflected a random component of error, which was independent of concentration, and for purposes of comparing the precision of the measurements in different plasma fractions, the CVs are specified for a particular concentration. The slope of variation (SV) is the slope of the regression line relating standard deviation to mean measured concentration, and describes that component of variability that was related to concentration. SV was calculated as: SV = (ΔSD measured concn) × 100.

Results

Quantitative Analyses

The concentrations of triglycerides, total cholesterol, HDL-cholesterol, and the cholesterol in the ultracentrifugal fraction of d >1.006 kg/L were determined by direct analysis
Table 1. Precision of Lipid and Lipoprotein-Cholesterol Measurements in 12 Lipid Research Clinics Laboratories

<table>
<thead>
<tr>
<th>Measured variables</th>
<th>Instrument</th>
<th>Mean (and 1 SD) b</th>
<th>Range of SD c</th>
<th>SD d at 0.50 g/L</th>
<th>CV e at 0.50 g/L</th>
<th>SD d at 2.00 g/L</th>
<th>CV e at 2.00 g/L</th>
<th>SV e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>AAII</td>
<td>1.933 (0.480)</td>
<td>0.012–0.099</td>
<td>0.050</td>
<td>2.5</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol in d &gt; 1.006 g/L fraction</td>
<td>AAII</td>
<td>1.512 (0.659)</td>
<td>0.010–0.085</td>
<td>0.065</td>
<td>4.4</td>
<td>2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>AAII</td>
<td>0.286 (0.118)</td>
<td>0.007–0.093</td>
<td>0.010</td>
<td>3.5</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>AAII</td>
<td>0.497 (0.349)</td>
<td>0.013–0.099</td>
<td>0.063</td>
<td>12.3</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>AAII</td>
<td>1.118 (0.565)</td>
<td>0.021–0.109</td>
<td>0.078</td>
<td>7.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>AAII</td>
<td>1.021 (0.304)</td>
<td>0.027–0.125</td>
<td>0.074</td>
<td>6.3</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AAII</td>
<td>0.353 (0.388)</td>
<td>0.024–0.090</td>
<td>0.048</td>
<td>13.6</td>
<td>1.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Samples 1–26 analyzed with AAII (n = 26), samples 15–53 with AAII (n = 39). b Group mean (and 1 SD) for 26 samples on AAII and 39 samples on AAII. c Range of standard deviations obtained for individual samples. d Predicted value, based on ordinary least squares regression line of standard deviation as a function of concentration within the observed range. e Underlined values are referred to in text. f NS, the slope of the regression line was not significantly different from zero. g ( ) obtained by extrapolation of observed range to 0.5 g/L.

and are referred to here as ‘measured variables.’ LDL- and VLDL-cholesterol concentrations were determined from the measured variables, and are referred to as ‘calculated variables.’ The results of the analyses are summarized in Table 1. The CVs for triglyceride measurements at 2.00 g/L were 5.0 and 5.7% for analyses performed with the AAII and AAII, respectively. The corresponding CVs for the total cholesterol measurements were 2.5 and 2.1%. In all cases the values agreed well with results reported previously on the basis of external-surveillance data (5,6).

The cholesterol concentrations in unfraccionated plasma, the ultracentrifugal infranatant fraction, and LDL were in the same general concentration range, but these successive measurements required a series of manipulations of increasing complexity. It was therefore of interest to compare the precision achieved for each of the three analyses. The CVs for the three analyses with the AAII were 2.1, 4.0, and 6.3% at 2.00 g/L (Table 1). The regression lines relating SD to cholesterol concentration (Figure 2a) were approximately parallel, but the intercepts increased with increasing complexity of the test. These results indicated that the magnitude of the concentration-dependent component of variability (SV) was the same for all three measurements. The SV for the analyses was approximately 1.5% (Table 1). The overall variability (CV) of the LDL-cholesterol measurements at 2.00 g/L was about threefold that for total cholesterol (Table 1), and the increase appeared to be attributable to the random component of variability, due presumably to manipulations required for the preparation of lipoprotein fractions. Similar results were obtained for the AAII measurements (Table 1).

VLDL- and HDL-cholesterol concentrations were in the same range, and it was of interest to compare their variabilities. The HDL measurements required precipitation with heparin–MnCl2 and one cholesterol measurement, whereas the VLDL measurements required ultracentrifugation and two cholesterol measurements (Figure 1). The CVs for the two analyses with the AAII were 8.7 and 13.6%, respectively, at 0.50 g/L (Table 1). The regression lines relating SD to cholesterol concentration are shown in Figure 2B. The regression line for VLDL-cholesterol had a positive slope and an SV of 1.8%. That for HDL-cholesterol had an SV not significantly different than zero (Table 1), indicating that the random component of variability accounted for essentially all of the imprecision of the measurements. The results for the AAII measurements were similar, except that the SVs for both HDL-cholesterol and VLDL-cholesterol did not differ significantly from zero.

The precision of the calculated variables can be conveniently viewed in terms of the precision of the summands used to compute them. Because each calculated variable was computed as the difference between two positively correlated measured variables, the variance of the differences was expected to be less than the sum of the variances of the two measured variables and weighted toward the less precise one. Indeed, this was observed. In addition, since in general, the

Fig. 2. Linear regressions relating standard deviation to cholesterol concentration in the measured fraction, for samples analyzed with the AAII

Ordinate reflects standard deviation about mean of analyses of each sample in all laboratories, n = 38; A, total cholesterol, SD = 0.0147 [total cholesterol] + 0.011; d > 1.006 kg/L fraction, SD = 0.0182 [d > 1.006 kg/L fraction] + 0.022; LDL-cholesterol SD = 0.0184 [LDL-cholesterol] + 0.041; 2, HDL-cholesterol, SD = 0.005 [HDL-cholesterol] + 0.033; VLDL-cholesterol, SD = 0.018 [VLDL-cholesterol] + 0.039. Regression lines are shown for the ranges of concentrations measured. All slopes and intercepts were significantly (p < 0.1) different from zero, except slope for HDL-cholesterol
Fig. 3. Linear regressions relating standard deviation of measured fraction to total cholesterol concentration, for samples analyzed with the AAI.

SD increases with cholesterol concentration, the precision of the calculated variables is dependent upon the proportion of total cholesterol that is being measured in each fraction. Figure 3A illustrates the relationship between SD and total cholesterol concentration for LDL-cholesterol, and the two measurements from which it was calculated. The data in this figure are from AAI analyses. The SD for LDL-cholesterol was similar to that of the $d > 1.006$ fraction, its most significant contributor. Similar results were obtained for AAI analyses. All pairwise differences between LDL-, ultracentrifugal infranatant-, and total cholesterol measurements were significant at the 0.1 level by the Wilcoxon signed ranks test (14), with the exception of the [total cholesterol – $d > 1.006$ fraction-cholesterol] difference for AAI measurements. The SD for VLDL-cholesterol was intermediate to those for the two relatively large and positively correlated variables from which it was calculated (Table 1). The differences between the precision of the VLDL- and HDL-cholesterol analyses were significant ($p < 0.1$) for both AAI and AAI1 (Figure 3B, AAI1 data).

Qualitative Determinations

Standing plasma test. Complete qualitative data were collected for 27 samples (samples 27–53), and the comparability of each of the four qualitative analyses was assessed by determining the extent to which the interpretations were unanimous. The findings are summarized in Tables 2 and 3.

The laboratories agreed unanimously that 21 (78%) of the 27 samples did not contain chylomicrons as judged by the standing plasma test (Table 2a). These samples had a mean triglyceride concentration of 1.40 g/L (range, 0.50–3.60 g/L). Four of the remaining six samples were judged to lack chylomicrons by over 70% of the laboratories. The triglyceride concentrations of these samples averaged 1.11 g/L (range, 0.70–1.45 g/L). The principal source of disagreement was the presence of a very slight film that was detected in some of the laboratories when the samples were agitated. The last two

Table 2. Interpretation of “Standing Plasma” Test in Lipid Research Clinics Laboratories

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>No samples</th>
<th>%</th>
<th>Triglyceride concn # (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples analyzed</td>
<td>27 b</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Absent: all laboratories</td>
<td>21</td>
<td>78</td>
<td>1.40 (0.89)</td>
</tr>
<tr>
<td>&gt;50% of laboratories</td>
<td>4</td>
<td>15</td>
<td>1.11 (0.34)</td>
</tr>
<tr>
<td>Present: ≥50% of</td>
<td>2</td>
<td>7</td>
<td>2.87 (0.01)</td>
</tr>
<tr>
<td>laboratories</td>
<td></td>
<td></td>
<td>Slightly turbid/turbid: ≥75%</td>
</tr>
<tr>
<td>Unanimous agreement</td>
<td>21</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>≥75% agreement</td>
<td>24</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>No samples</th>
<th>%</th>
<th>Triglyceride concn # (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear: all laboratories</td>
<td>10</td>
<td>37</td>
<td>0.92 (0.40)</td>
</tr>
<tr>
<td>&gt;50% of laboratories</td>
<td>14</td>
<td>52</td>
<td>1.50 (0.78)</td>
</tr>
<tr>
<td>Slightly turbid/turbid: ≥75% of laboratories</td>
<td>3</td>
<td>11</td>
<td>3.11 (0.42)</td>
</tr>
<tr>
<td>Unanimous agreement</td>
<td>10</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>≥75% agreement</td>
<td>23</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from overall laboratory mean levels for each sample. * Two of the 27 samples were analyzed in 11 laboratories; the remaining samples were analyzed in 12 laboratories.

Table 3. Interpretation of Lipoprotein Electrophoresis in Lipid Research Clinics Laboratories

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>No samples</th>
<th>%</th>
<th>Absent: all laboratories</th>
<th>15</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples analyzed</td>
<td>27 #</td>
<td>100</td>
<td>absent: all laboratories</td>
<td>15</td>
<td>56</td>
</tr>
<tr>
<td>Absent: all laboratories</td>
<td>17</td>
<td>63</td>
<td>&gt;50% of laboratories</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>&gt;50% of laboratories</td>
<td>3</td>
<td>11</td>
<td>Present: all laboratories</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Present: all laboratories</td>
<td>3</td>
<td>11</td>
<td>&gt;50% of laboratories</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Unanimous agreement</td>
<td>20</td>
<td>74</td>
<td>Unanimous agreement</td>
<td>15</td>
<td>56</td>
</tr>
<tr>
<td>≥75% agreement</td>
<td>25</td>
<td>93</td>
<td>≥75% agreement</td>
<td>19</td>
<td>70</td>
</tr>
</tbody>
</table>

* Two of the 27 samples were analyzed in 11 laboratories, the rest in 12 laboratories.
samples were reported to contain chylomicrons by six and 10 of the 12 laboratories, respectively; these samples had triglyceride concentrations of 2.86 and 2.88 g/L.

The laboratories reported unanimously that 10 (37%) of the samples had clear infranatant layers (Table 2b). The mean triglyceride concentration in these samples was 0.92 g/L (range, 0.50-1.66 g/L). Fourteen of the remaining 17 samples were judged to be clear by 55-92% of the laboratories, and the major source of disagreement was the assessment of slight turbidity in most of these samples by some of the laboratories. The mean triglyceride concentration in these samples was 1.50 g/L (range, 0.58-2.80 g/L). The last three samples were judged to be turbid by at least 75% of the laboratories, and had triglyceride concentrations of 2.86-3.80 g/L.

Lipoprotein electrophoresis. The laboratories unanimously agreed about the presence or absence of β-VLDL in 20 (74%) of the samples (Table 3). The seven samples on which agreement was incomplete were about equally divided between those for which the majority of laboratories assessed β-VLDL as present and those for which the majority did not detect β-VLDL.

The laboratories agreed completely on the absence of detectable "sinking prebeta" lipoproteins in 15 (56%) of the samples (Table 3). The major source of disagreement appeared to be in the observation of "sinking prebeta" lipoproteins by some laboratories in samples that most of the laboratories interpreted as "negative."

Discussion

The procedures used for determining lipoprotein cholesterol concentrations in plasma can be broadly divided into "separatory" steps and "analytical" steps. The separatory steps are the manipulations required to prepare the two ultracentrifugal fractions and the heparin–Mn²⁺ supernatant fraction, and include the measurement of sample and reagent volumes, preparation of reagents, centrifugation, and the recovery of the appropriate plasma fractions. The term "analytical steps" refers here to the preparation of zeolite-treated isopropanol extracts of the plasma fractions and the analysis of the extracts.

Thus, by definition, determination of total cholesterol and triglycerides required only the analytical steps. Furthermore, while the determination of cholesterol in plasma fractions required both separatory and analytical steps, the latter were identical to those used for the determination of total plasma cholesterol. The analytical steps were readily monitored with the aid of pooled serum control materials that were analyzed repeatedly by all the laboratories (4–8). It was expected, however, that the variability of the lipoprotein determinations would be greater than that of the lipid determinations because of the separatory steps required, and that the total variability would be underestimated by a system that considered only the analytical steps. In the absence of appropriate quality-control materials, this issue was addressed by introduction of a surveillance procedure in which aliquots of freshly drawn plasma samples were sent periodically to all the laboratories for analysis. All of the laboratories analyzed each sample once and the results, therefore, reflect the total variability of the analyses in all the laboratories; no attempt was made to separate inter- from intra-laboratory variation. The number of samples analyzed was relatively small, owing primarily to logistical limitations, and the estimates of precision are consequently less certain than those reported previously for total cholesterol and triglyceride analyses (3–6). The precision of the total cholesterol and triglyceride measurements in the present study, however, were similar to those reported previously, and the observed variabilities of the other measurements therefore are probably fairly representative of the degree of precision attained for the lipoprotein analyses during the 4½-year period of the study.

Since the precision of an assay decreases as the number of required manipulations increases, it was of interest to examine the extent to which this factor influenced the present results. It was possible to make a more or less direct comparison of the precision of the cholesterol values for unfraccionated plasma, the ultracentrifugal infranatant fraction, and LDL-cholesterol, because all three measurements span approximately the same range of cholesterol concentration. Because all three analyses require the same analytical steps, the difference between the precision of the total cholesterol analyses and those of the other two analyses can be used to estimate the variability introduced by the additional manipulations. Using the CV at 2.00 g/L as a measure of overall precision, the variability of the ultracentrifugal infranatant analyses was about twice that of total cholesterol, and the variability of LDL-cholesterol was about three times as great. The concentration-dependent component of variability was about the same for all three parameters. Overall, the findings indicated that this component was determined almost entirely by the analytical steps, and that the increased variability of the >1.006 kg/L fraction, and LDL-cholesterol values was largely ascribable to the contribution of the separatory steps to the random-error component of the analyses.

The data in the present study do not permit a determination of the extent to which the separatory steps contributed to the variability of HDL- and VLDL-cholesterol analyses, because the precision of the analytical steps in the low-concentration range was not directly measured. Assuming, however, that the precision of the analytical steps can be estimated by extrapolation of the regression line for total cholesterol (Figure 2A), the CV at 0.50 g/L for HDL- and VLDL-cholesterol measurements would be about 2.5 and 3.5 times greater than accounted for by the analytical steps. As with LDL-cholesterol, most of the variation seemed to result from random errors contributed by the separatory steps. In any case, the more involved determination of VLDL-cholesterol was clearly less precise than that of HDL-cholesterol.

The limitations of the present study preclude accurate numerical estimates of the contribution of the separatory steps to the overall variability of the analysis of each lipoprotein class, but it is probably reasonable to conclude that the variability of the lipoprotein analyses, with a relative measure such as CV as an indicator, was at least three or four times that of the total cholesterol measurements.

The interpretation of qualitative tests for components that can vary in concentration depends on the component concentrations, the sensitivity of the methods used, the experience and judgment of the technician performing the tests and, with lipoproteins, the nature and stability of the lipoproteins themselves. In the present case, different factors affected the qualitative detection of chylomicrons, turbidity, β-VLDL, and sinking prebeta lipoproteins. For example, detection of chylomicrons would be expected to be affected primarily by their concentration. The detection of the turbidity in the infranatant fraction of standing plasma, which is generally taken to indicate high concentrations of VLDL, might also be affected by the presence of protein aggregates unrelated to VLDL. Likewise, the detection of β-VLDL and "sinking prebeta" lipoproteins in the ultracentrifugal fractions will depend not only on their concentrations, but also on how well they were resolved by the electrophoretic procedures used, the sensitivity of the staining procedure, and other factors. It was not surprising, therefore, that the evaluations of the four qualitative tests varied considerably in some samples.

One measure for comparing the relative performance of the four tests was simply to examine how often there was una-
nimity of interpretation for each. Such an examination revealed some interesting trends. The laboratories completely agreed on the interpretation of the chylomicron test in 78% of the samples, all of which lacked chylomicrons. Most of the disagreement arose from the interpretation of the presence of chylomicrons by some laboratories in samples which the majority of the laboratories considered negative. Assuming that the majority finding is the most likely one, the test might be considered prone to false positives.

There was less agreement among laboratories about the interpretation of the turbidity test; agreement was complete for 37% of the samples. Disagreement was most often due to the detection of turbidity in samples that most of the laboratories judged to be clear; this type of disagreement occurred in 52% of the samples, over half of which had triglyceride concentrations of <1.50 g/L. Again, if it is assumed that the majority finding is the most likely, this test also seemed to be prone to false positives.

There was complete agreement about the presence or absence of sinking prebeta lipoproteins for 56% of the samples. Again, the major source of disagreement (37% of the samples) was the interpretation of samples that most of the laboratories considered negative; the opposite interpretation was made for 7% of the samples. The laboratories agreed about the presence or absence of \( \beta \)-VLDL in 74% of the samples. There seemed to be an approximately equal incidence of false positives and false negatives with this test.

Overall, the tests for chylomicrons and \( \beta \)-VLDL appeared to be the least difficult of the four to interpret. At least three-quarters of the laboratories agreed on the interpretation of both of these tests in 93% of the samples.

It should be mentioned that the observations made in the present study relate to the variability of interpretation of the qualitative tests, and not to how accurately they reflect the parameters they are intended to measure. An assessment of the latter requires the measurement of the four variables by independent methods, rather than by consensus; the terms “false positive” and “false negative” have therefore been used as terms of convenience to summarize trends in the observed disagreement. We also stress that the samples used for this study were from patients who were selected because they were marginally hyperlipidemic, or who were under treatment at the time of sampling. The interpretation of the qualitative tests, particularly the “standing plasma” test, probably reflected a greater degree of variability than would be encountered when applied for the usual screening or diagnostic purposes.

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