Five Methods for Measuring Low-Density Lipoprotein Cholesterol Concentration in Serum Compared

G. E. Hoffmann, R. Hieflinger, L. Weis, and W. Poppe

Five methods for the quantification of low-density-lipoprotein cholesterol were compared: ultracentrifugation, electrophoresis, precipitation with polyvinyl sulfate or heparin, and an indirect calculation procedure (Friedewald formula). Excellent agreement of results was obtained with all procedures for 49 of 51 sera. Discrepancies were as much as 1.69 g/L for the remaining two cases, which contained appreciable amounts of "floating" beta-cholesterol as detected with a combination of ultracentrifugation and electrophoresis.

Additional Keyphrases: ultracentrifugation · electrophoresis · polyanion precipitation · "floating beta" lipoprotein

Recently, two practicable methods have been described by which one can determine low-density-lipoprotein cholesterol concentrations in serum after precipitation of the LDL with polyonions such as polyvinyl sulfate (1) or heparin (2). Here, we compare results of these tests with those of such well-established procedures as ultracentrifugation (3, 4) and quantitative electrophoresis (5), and focus on discrepant results of the different methods.

Materials and Methods

Using sera from fasting subjects, submitted to the laboratory for routine clinical examination, we determined concentrations of triglycerides (6) and cholesterol (7) and the HDL, VLDL, and LDL subfractions of serum cholesterol. To measure the lipoprotein subfractions, we used the following methods:

VLDL and LDL were precipitated by adding 500 µL of phosphotungstic acid/MgCl₂ (1.6 g/L) to 200 µL of the sample. The solution was mixed, incubated for 15 min at room temperature, then centrifuged for 2 min at 12 000 × g (8, 9). LDL-cholesterol was determined by measuring cholesterol with an enzymatic procedure (Boehringer Mannheim GmbH, Mannheim, F.R.G.) in the supernate. The concentration of LDL-cholesterol was calculated according to Friedewald et al. (10):

\[ \text{LDL-chol.} = \text{total chol.} - \left( \text{triglycerides/5} \right) - \text{HDL-chol.} \]

LDL were precipitated by adding 100 µL of polyvinyl sulfate (1 g/L) to 200 µL of the sample. The solution was mixed, incubated for 15 min at room temperature, then centrifuged for 2 min at 10 000 × g (1). The concentration was calculated from the differences between the total serum cholesterol and the cholesterol in the supernate (PVS method, Boehringer Mannheim). With an analogous procedure (2) LDL were precipitated by adding 1000 µL of heparin (0.68 g/L, 100 000 int. units/L; Merck, Darmstadt, F.R.G.) to 100 µL of the sample.

Alpha-, pre-beta-, and beta-cholesterol concentrations were determined after electrophoresis and precipitation of the separated bands (9), with the test combination and equipment from Immuno, Heidelberg, F.R.G. We used a nomogram (II) to evaluate the densitometer readings; the concentration of beta-cholesterol was taken as a measure for LDL-cholesterol.

Ultracentrifugation of VLDL by ultracentrifugation was as previously described (3, 4). We used a air-driven centrifuge ("Airfuge"; Beckman Instruments, Fullerton, CA) to centrifuge 170 µL of serum for 180 min at 200 000 × g, the VLDL-containing supernates (50 µL) were separated by use of a special cutting instrument (Beckman Instruments; cat. no. 924116). Upper and lower layers so obtained were used in determining cholesterol and triglyceride concentrations with enzymatic procedures (Boehringer Mannheim). HDL-cholesterol was determined in the infranatant layer after precipitation (8, 9). To detect floating beta-lipoproteins in the separated fractions, we checked super- and infranatants by quantitative electrophoresis and, if necessary, corrected for contamination.

All analyses described were run in duplicate and mean values were used for statistical evaluation.

Results

In the present study we analyzed 51 sera from fasting subjects whose triglyceride concentrations were below 4 g/L. The coefficients of variation (CVs) obtained in the repeat analyses were between 2.6 and 3.6% for cholesterol and triglycerides and between 3.2 and 6.1% for HDL- and LDL-cholesterol (Table 1). CVs were particularly low (about 2%) for the electrophoretic determination of LDL-cholesterol (duplicate analyses performed on the same plate).

Two sera that contained abnormal distributions of lipoprotein subfractions (see below) were excluded from the statistical analyses.

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<th>Table 1. Precision Data for the Various Methods</th>
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*Calculated from duplicate assays (13). **No. pairs.
The remaining 49 sera showed high correlation coefficients (0.90 to 0.98) for intercomparisons of the five LDL-cholesterol methods. The best correlation (r = 0.98) was between the results by the Friedewald formula vs PVS precipitation—although 37 of the 49 values were lower by the calculation of Friedewald et al. (10).

The results obtained with the other four LDL-cholesterol methods agreed well with those by the ultracentrifugation method, especially the PVS precipitation method (Figure 1; r = 0.94) and quantitative electrophoresis (r = 0.95). The latter gave slightly higher values than did ultracentrifugation (median differences = 0.13 g/L), with greater discrepancies at low concentrations of LDL-cholesterol.

In the two cases not included in Figure 1, unexpectedly high discrepancies were found between the results of the different LDL-cholesterol methods. We obtained the lowest values with the PVS method (1.59 and 1.97 g/L), and the highest ones with electrophoresis (3.28 and 3.30 g/L). The results obtained with ultracentrifugation were slightly lower (3.10 and 2.85 g/L). The supernates from the two sera after ultracentrifugation showed unusually high concentrations of floating beta-cholesterol (0.23 and 0.35 g/L). These values were two to three times higher than the corresponding pre-beta-cholesterol concentrations in the supernates.

Discussion

Given the great clinical importance of LDL-cholesterol (10), the development of simple, routine methods for its accurate quantification is in urgent demand. The most commonly used reference method is preparative ultracentrifugation (14), which is, however, too time-consuming and laborious for routine purposes. Our studies confirm that the simplified ultracentrifugation procedure (3, 4) offers a useful alternative if the homogeneity of the super- and infranates is checked by electrophoresis (5).

Precipitation techniques (1, 2) has greatly simplified the direct quantification of LDL-cholesterol. If sera with triglyceride concentrations exceeding 4.0 g/L are excluded from routine analysis, the results obtained with these precipitation methods agree with the more conventional Friedewald calculation method, usually within 0.2 g/L.

In cases where the results differ by more than 0.2 g/L, additional examination by electrophoresis (5) or ultracentrifugation (3, 4, 13) should be used to check for the presence of abnormal lipoproteins—as we found in two of 51 cases.

References