Analytical and Clinical Performance of a Detergent-based Homogeneous LDL-Cholesterol Assay: A Multicenter Evaluation

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Background: LDL-cholesterol (LDL-C) concentrations currently are determined in most clinical laboratories using the Friedewald calculation. This approach has several limitations and may not always meet the current total error recommendation in LDL-C measurement of ≤12% established by the National Cholesterol Education Program.

Methods: In a multicenter study, we evaluated the analytical and clinical performance of a homogeneous LDL-C assay (LDL-C_Roche; Roche Diagnostics, Indianapolis, IN) in a comparison with a β-quantification method.

Results: This direct assay correlated highly with a β-quantification method (r = 0.968; y = 1.037x – 95.8 mg/L; n = 355; 95% confidence intervals, 1.011–1.063 for the slope and –129.5 to 62.0 mg/L for the y-intercept) and met the current total error requirement. The assay was not affected significantly by concentrations of hemoglobin up to 6000 mg/L or bilirubin up to 500 mg/L. However, a negative bias of 10% was seen when triglyceride concentrations exceeded 10 000 mg/L. At the medical decision cut-point range, the LDL-C_Roche assay showed positive predictive values of 91–100% and negative predictive values of 80–99%. Furthermore, the clinical utility of the assay seemed unaffected in samples obtained postprandially.

Conclusions: The homogeneous LDL-C_Roche assay meets the currently established analytical performance goals and may be useful for the diagnosis and management of hyperlipidemic patients.

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calculation (LDL-C_{Fried}), which is the most commonly used procedure in clinical laboratories for the estimation of LDL-C, is the routine method recommended by the NCEP Working Group on Lipoprotein Measurement (6, 7). Although the latter method correlates highly with the β-quantification, it has several shortcomings (8): it is invalid when a specimen is collected in the nonfasting state or from a patient with type III hyperlipoproteinemia, or in the presence of increased triglycerides (TGs >4000 mg/L). Furthermore, because this calculation requires the determination of three different measurements [TC, TGs, and HDL-cholesterol (HDL-C)], each with its own analytical CV, it may not always meet the performance criteria of total error ≤12% established by the NCEP. Therefore, the NCEP Working Group on Lipoprotein Measurement recommended the development of direct methods for LDL-C measurement (7). A new homogeneous assay for the determination of LDL-C has been developed recently and is being introduced by Roche Diagnostics (LDL-C_{Roche}, Roche Diagnostics, Indianapolis, IN) (9). The goal of this multicenter study was to evaluate the analytical and clinical performance of this new assay.

**Materials and Methods**

**SAMPLES**

Fresh serum samples (n = 355) were obtained, after an overnight fast, at three centers for the patient correlation studies. Paired serum samples, one obtained after 12-h fasting and another 3.5 h after a high-fat meal (~32 g of fat), were collected from 43 healthy subjects to determine the postprandial effect on the measurement of LDL-C by this direct homogeneous method. In addition, 29 fresh-frozen human serum samples were sent to seven laboratories in the United States and Europe, where the homogeneous LDL-C_{Roche} assay was performed using the Roche/Hitachi 704, 911, 912, or 917 analyzers. These samples were characterized by the LDL-C_{CRMLN} method at Northwest Lipid Research Laboratories in Seattle, WA, a Cholesterol Reference Method Laboratory Network (CRMLN) Laboratory.

**LIPID MEASUREMENTS**

TC and TGs were determined enzymatically with the CHOD-PAP (cat. no. 450061; Roche Diagnostics) and GPO-PAP (cat. no. 1488872; Roche Diagnostics) methods, respectively, according to the manufacturer’s specifications. The day-to-day imprecision of the two methods, reflected by the CV when Precinorm® L and Precipath® HDL/LDL controls were used, was <3%. HDL-C was measured using a homogeneous assay (cat. no. 1930672; Roche Diagnostics) (10–13). HDL-C was measured with a day-to-day CV of <3%.

**LDL-C_{Roche} ASSAY**

At neutral pH (pH 7.0) and in the presence of MgCl₂, sulfated α-cyclodextrin, and dextran sulfate, the enzymatic reaction for cholesterol in VLDL and chylomicrons is markedly reduced (reagent 1, cat. no. 1985604; Roche Diagnostics). The nonionic detergent in reagent 2, which selectively solubilizes LDL-C but not HDL-C, enables the measurement of LDL-C by a conventional enzymatic reaction (9). The assay was calibrated as recommended with the Calibrator for automated systems (C.f.a.s.) LDL-C Plus calibrator, which is standardized to the CRMLN reference method, and performed according to the manufacturer’s recommendation.

**β-QUANTIFICATION (LDL-C_{UC})**

An accurately measured volume of serum was placed into an ultracentrifugation tube, overlayed with sufficient 0.15 mol/L NaCl to fill the tube, and centrifuged at 105 000g for 18 h at 10 °C in Baltimore and St. Louis. In Boston, ultracentrifugation was performed at 250 000g for 3 h at 10 °C. The floating layer containing VLDL and chylomicrons (if present) was removed, and the infranatant was reconstituted to known volume and analyzed for cholesterol using the enzymatic cholesterol assay. LDL-C_{UC} was calculated as the difference between the cholesterol concentration of the infranatant and HDL-C, measured by dextran sulfate precipitation in Boston and St. Louis and by homogeneous assay in Baltimore. All three laboratories that performed the β-quantification are certified by the CDC National Heart, Lung and Blood Institute Lipid Standardization Program (TC, TG, HDL-C) and two of them participate in the Alert Proficiency Survey (Pacific Biometrics Research Foundation, Seattle, WA) to periodically check the accuracy of the β-quantification procedure. The LDL-C concentrations of the 29 samples involved in the interlaboratory survey were determined by the CRMLN Method (LDL-C_{CRMLN}), which uses a heparin/Mn²⁺ precipitation with subsequent cholesterol determination by the Abell-Kendall method.

\[
LDL-C_{Fried} = [TC] - [HDL-C] - [TG]/5
\]

after excluding samples with TGs >4000 mg/L. [TG]/5 is an estimate of VLDL-C. All concentrations are in mg/L.

**LINEARITY**

Human LDL concentrates (Scantibodies, Inc.) were serially diluted with 9 g/L NaCl at 11 different concentrations by the eight participating laboratories. The predicted values were calculated by a regression line according to Passing and Bablok, using LDL-C_{Roche} concentrations up to 4000 mg/L (14).

**INTERFERENCES**

Interferences from hemoglobin and bilirubin were determined according to Glick et al. (15). In addition, isolated VLDL and chylomicrons were added to different pooled
sera at various concentrations to determine the effect of increased TGs on the measurement of LDL-C<sub>Roche</sub>.

**METHOD COMPARISON**

In three laboratories, the LDL-C<sub>Roche</sub> assay and the LDL-C<sub>Fried</sub> were compared with the LDL-C<sub>UC</sub>. A total of 355 fresh sera were analyzed in parallel. In addition, the influences of increasing concentrations of LDL-C<sub>UC</sub> and TGs on the LDL-C<sub>Roche</sub> assay were examined by bias plots using the 355 samples mentioned above. To account for the interlaboratory biases of the lipid determinations in the three laboratories, the data were adjusted to CDC reference values using the results of CDC National Heart, Lung and Blood Institute Lipid Standardization Program Part III measurements made in the three laboratories during the study period January 1998 through March 1998. In each laboratory, four different samples for the lipid measurements of TC, TGs, and HDL-C were analyzed 18 times. The biases for TC, TGs, and HDL-C were calculated for each sample, using the differences of the results by the CDC and each laboratory; the mean biases were calculated for each sample, using the differences of the results by the CDC and each laboratory; the mean biases for TC, TGs, and HDL-C were then calculated for each laboratory. The mean biases were −43 to 17 mg/L for TC, −12 to 24 mg/L for TGs, and 2–11 mg/L for HDL-C, respectively.

The adjustments of the data were then calculated specifically for each laboratory. The adjustments for LDL-C<sub>UC</sub> and LDL-C<sub>Fried</sub> were performed according to the following equations:

\[
\text{Adjusted LDL-C}_{\text{UC}} = \text{Observed LDL-C}_{\text{UC}} + \text{Bias}_{\text{TC}} - \text{Bias}_{\text{HDL-C}}
\]

\[
\text{Adjusted LDL-C}_{\text{Fried}} = \text{Observed LDL-C}_{\text{Fried}} + \text{Bias}_{\text{TC}} - \text{Bias}_{\text{HDL-C}} - \left(\frac{\text{Bias}_{\text{TC}}}{5}\right)
\]

These adjusted data were used for all analyzes.

**PRECISION STUDY**

Two commercial control sera, with low and high LDL-C concentrations, and two human serum pools were used to assess the precision of the new homogeneous LDL-C assay in eight laboratories, according to the NCCLS EP5-T protocol (16).

**POSTPRANDIAL STUDY**

In this study, the effect of feeding on the determination of LDL-C concentration by the Roche assays was examined in paired samples from 43 subjects.

**STORAGE OF SAMPLES**

Fresh serum samples (n = 125) were assayed using the LDL-C<sub>Roche</sub> assay immediately after collection and after storage at −20 °C for up to 12 months in a noncycling freezer to determine the effect of storage on the measurement of LDL-C<sub>Roche</sub>. In addition, the short-term storage up to 14 days at 4 °C was also examined using two serum pools.

**STATISTICAL METHODS**

Regression analyses were performed using the method of Passing and Bablok (14). Total error was calculated as the sum of the systematic error plus random error (17, 18). Systematic error was calculated from the linear regression equation \( y_c = bx_c + a \), where \( b \) is the slope of the regression line, and \( a \) is the y-axis intercept (LDL-C<sub>UC</sub> vs LDL-C<sub>Roche</sub>). At an LDL-C concentration of \( x_c \), systematic error was the absolute value of \( y_c - x_c \). Random error was \( 1.96 \times CV \), based on the run-to-run precision study. Results were considered statistically significant at \( P < 0.05 \). The positive predictive value (PPV) of an LDL-C assay at each specified cut-point was calculated as \( \left[ \frac{\text{true positive}}{\text{true positive} + \text{false positive}} \right] \times 100 \), where “true positive” means that the LDL-C results of both the comparison procedure (LDL-C<sub>UC</sub>) and the test method (LDL-C<sub>Roche</sub> or LDL-C<sub>Fried</sub>) were greater than or equal to the cutoff concentration, and “false positive” means that the test method LDL-C result was greater than the cut-point when the reference procedure LDL-C value was less than the cut-point. The negative predictive value (NPV) of an LDL-C assay at each specified cut-point was calculated as \( \left[ \frac{\text{true negative}}{\text{true negative} + \text{false negative}} \right] \times 100 \), where “true negative” means that the LDL-C results of both the reference procedure and the test method were less than the cut-point concentration, and “false negative” means that the test method LDL-C result was less than the cut-point when the reference procedure LDL-C value was greater than or equal to the cut-point concentration.

**Results**

**ANALYTICAL PERFORMANCE**

Two lyophilized control sera (Precinorm L and Precipath HDL/LDL) and two human serum pools, prepared by the individual sites, were used to assess the imprecision of the LDL-C<sub>Roche</sub> assay in all eight participating laboratories. No difference in the reproducibility was observed between the lyophilized controls and the human serum pools. The mean total CVs at LDL-C concentrations of ~700 mg/L (human serum pool 1), 950 mg/L (Precinorm L), 1300 mg/L (human serum pool 2), and 2100 mg/L (Precipath L) were between 0.7% and 3.1% (Table 1) and did not vary significantly with concentration.

**LINEARITY**

The dilution experiment conducted in all participating laboratories demonstrated that the assay is linear up to 4000 mg/L of LDL-C \( y = 0.996x - 1.29 \text{ mg/L}; r = 1.000; n = 52; 95\% \text{ confidence interval (CI), 0.989–1.002 for the slope, and −7.26 to 1.28 mg/L for the y-intercept} \). At LDL-C concentrations >4000 mg/L, negative biases from the expected values of approximately −5%, −9%, and
12% were seen at 5000, 6000, and 7000 mg/L, respectively.

METHOD COMPARISONS
A total of 355 freshly collected sera were analyzed in three laboratories with the LDL-CUC, the LDL-CRoche assay, and the LDL-C Fried calculation. Means, standard deviations (± 1 SD), and ranges for TC, TGs, HDL-C, and LDL-C UC were 2195 ± 494 (990–3880), 2017 ± 1632 (180–12 290), 470 ± 133 (189–1081), and 1357 ± 436 (240–2758) mg/L, respectively. The comparison-of-methods plot [LDL-C UC (x) vs test method (y)] showed regression lines according to Passing and Bablok of $y = 1.037x - 95.8$ mg/L ($r = 0.968$; $n = 355$; 95% CI, 1.011–1.063 for the slope, and $-129.5$ to 62.0 mg/L for the $y$-intercept) for the LDL-C Fried calculation (Fig. 1). For the row data, the equations of the regression lines according to Passing and Bablok were: (A), $y = 1.042x - 124.2$ mg/L ($r = 0.961$; $n = 355$; 95% CI, 1.011–1.072 for the slope, and $-158.4$ to 81.1 mg/L for the $y$-intercept) for the LDL-C Roche assay; and (B), $y = 1.025x - 69.0$ mg/L ($r = 0.974$; $n = 313$; 95% CI, 1.002–1.046 for the slope, and $-98.3$ to 40.6 mg/L for the $y$-intercept) for the LDL-C Fried assay (data not shown). In these method comparisons, the LDL-C Fried concentrations were included only for those samples with TG concentrations ≥4000 mg/L. To better illustrate the performance of the LDL-C Roche assay in hypertriglyceridemic samples, specimens with TG concentrations ≥4000 mg/L and those with TG concentrations ≤4000 mg/L were compared separately with the

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### Table 1. Summary of the precision studies in human serum pools prepared by the individual laboratories and common lyophilized control materials.a

<table>
<thead>
<tr>
<th>Sample</th>
<th>LDL-C concentration, mg/L</th>
<th>CV, %</th>
<th>Within-run</th>
<th>Day-to-day</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Low HSPb</td>
<td>683.3</td>
<td>433.5–856.7</td>
<td>0.89</td>
<td>0.40–1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Medium HSP</td>
<td>1356.2</td>
<td>1222.7–1630.0</td>
<td>0.63</td>
<td>0.30–0.80</td>
<td>1.5</td>
</tr>
<tr>
<td>Precinorm L</td>
<td>954.5</td>
<td>899.3–995.5</td>
<td>0.73</td>
<td>0.50–1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Precipath LDL/HDL</td>
<td>2138.4</td>
<td>2090.8–2190.8</td>
<td>0.65</td>
<td>0.30–1.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

a At all sites identical lots of the lyophilized control sera Precinorm L and Precipath LDL/HDL were used. All samples were analyzed with the homogeneous LDL-CRoche assay. Pools were divided into 300 μL aliquots and stored at 4 or –20 °C until tested. One aliquot of each pool was assayed in duplicate on each of 21 days. Calculations for within-run CVs were also based on 21 samples.

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b HSP, human serum pool.
LDL-C_{UC} procedure. A regression line of $y = 1.035x - 111.0$ mg/L ($r = 0.957; n = 313; 95\% CI, 1.002–1.068$ for the slope, and $-151.1$ to $68.4$ mg/L for the $y$-intercept) was obtained for samples with TG concentrations $<4000$ mg/L; and a line of $y = 1.074x - 169.5$ mg/L ($r = 0.954; n = 42; 95\% CI, 0.957–1.188$ for the slope, and $-293.9$ to $44.3$ mg/L for the $y$-intercept) was obtained for samples with TG concentrations $\geq 4000$ mg/L.

CONCENTRATION DIFFERENCE PLOTS

The concentration differences of the homogeneous LDL-C_{Roche} assay from LDL-C_{UC} and LDL-C_{Fried} from LDL-C_{UC} were examined as a function of either increased LDL-C_{UC} or TGs. In samples with TG concentrations $<4000$ mg/L, the LDL-C_{Roche} assay and the LDL-C_{Fried} showed a relatively constant mean negative bias of $-60$ and $-30$ mg/L, respectively, independent of the LDL-C_{UC} concentration. In samples with TG concentrations $\geq 4000$ mg/L, the scattering of LDL-C_{Roche} was comparable to the results of samples with TG concentrations $<4000$ mg/L, whereas the concentration differences increased tremendously for LDL-C_{Fried} (Fig. 2, A and B). The negative bias of the LDL-C_{Roche} assay increased slightly from a mean of $-56$ mg/L at a TG concentration of 1000 mg/L to a mean of $-82$ mg/L at a TG concentration of 10 000 mg/L. The mean bias of $-30$ mg/L of LDL-C_{Fried} was only marginally influenced by increasing TG concentrations up to 4000 mg/L. However, this negative bias increased dramatically in samples with TG concentrations $\geq 4000$ mg/L: up to more than $-500$ mg/L at a TG concentration of 10 000 mg/L (Fig. 2, C and D).

TOTAL ERROR

The systematic error of the LDL-C_{Roche} assay at various LDL-C concentrations, encompassing the clinical decision cut-points (1000–1900 mg/L), ranged from 0.8% to 6.3% (Table 2). The random error was always $<4\%$, as recommended by the NCEP (7). This assay fulfilled the current NCEP total error requirements for LDL-C at the clinical decision cut-point range.

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**Fig. 2.** The effect of increased LDL-C_{UC} and TG concentrations on the LDL-C measurement by the homogeneous Roche assay (A and C) and the Friedewald equation (B and D).

Concentration difference [test method LDL-C minus the LDL-C_{UC}] is plotted as a function of LDL-C_{UC} and TG concentrations, respectively. □, samples with TGs $<4000$ mg/L; ■, samples with TGs $\geq 4000$ mg/L, measured by the LDL-C_{Roche} assay, ○, samples with TGs $\geq 4000$ mg/L, estimated by the Friedewald equation (6). This calculation is not recommended for samples with TGs $\geq 4000$ mg/L. The dotted lines represent the absence of bias. The solid lines represent the regression of all samples measured by the LDL-C_{Roche} assay, or samples with TGs $<4000$ mg/L estimated by LDL-C_{Fried}. The dashed lines represent the regression line of samples with TGs $\geq 4000$ mg/L, estimated by LDL-C_{Fried}. CP, common precipitation.
Table 2. Total error of the new homogeneous LDL-C<sub>Roche</sub> assay as calculated at mean LDL-C concentrations that encompassed the clinical decision cut-points (1000, 1300, 1600, and 1900 mg/L).<sup>a</sup>

<table>
<thead>
<tr>
<th>LDL-C, mg/L</th>
<th>Systematic error, %</th>
<th>Run-to-run random error, %</th>
<th>Total error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>955</td>
<td>6.3</td>
<td>3.1</td>
<td>9.4</td>
</tr>
<tr>
<td>1356</td>
<td>3.4</td>
<td>2.9</td>
<td>6.3</td>
</tr>
<tr>
<td>2158</td>
<td>0.8</td>
<td>3.3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>The pools were the same as in Table 1.

INTERLABORATORY SURVEY
The LDL-C<sub>Roche</sub> values of the 29 fresh-frozen serum samples reported by seven participating laboratories were compared with those obtained by the LDL-C<sub>CRMLN</sub> method (Table 3). Means, standard deviations (± 1 SD), and ranges for TC, TGs, HDL-C, and LDL-C<sub>CRMLN</sub> were 2186 ± 381 (1290–2950), 1313 ± 788 (520–3510), 529 ± 160 (303–973), and 1427 ± 348 (690–2080) mg/L, respectively. The slopes and <i>y</i>-intercepts of the regression lines according to Passing and Bablok were comparable. All of the slopes were >1, whereas the <i>y</i>-intercepts were negative. The mean biases ranged from −12 to 72 mg/L. When the imprecision from these measurements was calculated, the interlaboratory CVs of the samples were on average 2.5%. These findings confirm the good agreement among the seven laboratories in the measurement of the LDL-C<sub>Roche</sub> assay, regardless of the type of the Roche/Hitachi analyzer used.

INTERFERENCES
Hemoglobin at a concentration of 6000 mg/L caused a positive bias in the LDL-C<sub>Roche</sub> assay by ~10% (data not shown). Unconjugated and conjugated bilirubin added to serum pools up to concentrations of 500 and 250 mg/L, respectively, showed a tendency to decrease the LDL-C<sub>Roche</sub> value by <5% (data not shown). Isolated VLDL and chylomicrons, which were added to serum pools at different concentrations, showed a negative bias slightly >10% when the TG concentration exceeded 10 000 mg/L (data not shown).

Table 3. Interlaboratory survey of 29 fresh-frozen human serum samples.<sup>a</sup>

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Analyzer</th>
<th>r</th>
<th>Slope</th>
<th>y-Intercept, mg/L</th>
<th>Mean bias, mg/L</th>
<th>Maximum negative bias, mg/L</th>
<th>Maximum positive bias, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltimore</td>
<td>Hi&lt;sup&gt;a&lt;/sup&gt; 704</td>
<td>0.989</td>
<td>1.047</td>
<td>−41.9</td>
<td>31.6</td>
<td>−66.8</td>
<td>191.1</td>
</tr>
<tr>
<td>Boston</td>
<td>Hi 911</td>
<td>0.981</td>
<td>1.048</td>
<td>−41.3</td>
<td>33.2</td>
<td>−104.3</td>
<td>166.0</td>
</tr>
<tr>
<td>Reims</td>
<td>Hi 911</td>
<td>0.990</td>
<td>1.110&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−81.7</td>
<td>71.57</td>
<td>−51.5</td>
<td>232.4</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>Hi 911</td>
<td>0.989</td>
<td>1.045</td>
<td>−63.3</td>
<td>6.1</td>
<td>−101.7</td>
<td>203.0</td>
</tr>
<tr>
<td>St. Gallen</td>
<td>Hi 917</td>
<td>0.989</td>
<td>1.059</td>
<td>−75.5</td>
<td>6.1</td>
<td>−95.9</td>
<td>105.4</td>
</tr>
<tr>
<td>St. Louis</td>
<td>Hi 917</td>
<td>0.991</td>
<td>1.048&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−46.8</td>
<td>30.78</td>
<td>−57.8</td>
<td>198.8</td>
</tr>
<tr>
<td>Verona</td>
<td>Hi 912</td>
<td>0.990</td>
<td>1.043</td>
<td>−67.6</td>
<td>−11.96</td>
<td>−108.9</td>
<td>108.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Correlation coefficients and regression lines for the homogeneous assay vs the LDL-C<sub>CRMLN</sub> method were calculated using the nonparametric method described by Passing and Bablok (14).

<sup>a</sup>Hi, Hitachi.

<sup>c</sup>Significantly different from unity: <i>P</i> < 0.05.

CLASSIFICATION OF PATIENTS ACCORDING TO NCEP GUIDELINES
The LDL-C<sub>Roche</sub> assay and the LDL-C<sub>Fried</sub> method were compared for their ability to appropriately classify patients into treatment groups as established by the NCEP (Fig. 3) (4). For this purpose, the UC method used in the three laboratories involved in the method comparison, adjusted to CDC reference values, was considered the reference method. In those subjects with LDL-C below 1000 and 1300 mg/L, 96% were classified correctly using the LDL-C<sub>Roche</sub> assay. In those with LDL-C concentrations of 1300–1600 and >1600 mg/L, 68–81% of subjects were classified correctly by the homogeneous method. The LDL-C<sub>Fried</sub> correctly classified a slightly higher percentage of subjects than the homogeneous method. It is important to note, however, that with the Friedewald method, only those samples with TG concentrations <4000 mg/L were included in the analysis. In those with LDL-C >1900 mg/L, both methods classified at least 89% of subjects correctly.

The effects of TGs on the classification of subjects into NCEP cut-points, using both the LDL-C<sub>Roche</sub> and LDL-C<sub>Fried</sub> methods, are presented in Table 4. The classification in samples with TG concentrations of <2000 mg/L and 2000–4000 mg/L was slightly better when the LDL-C<sub>Fried</sub> was used (88.6% vs 85.3% and 92.9% vs 88.9%). In samples with TG concentrations >4000 mg/L, for which the Friedewald calculation could not be used, the percentage of correctly classified subjects by the LDL-C<sub>Roche</sub> was nearly perfect (97.5% for TG concentrations of 4000–6000 mg/L, and 100.0% for TG concentrations >6000 mg/L).

PPV AND NPV
The ability of the LDL-C<sub>Roche</sub> assay to correctly classify subjects at the medical decision cut-points was evaluated in this study population (n = 355), using the LDL-C<sub>UC</sub> concentrations as the true values. For the interest of comparison, the PPV and NPV of LDL-C<sub>Fried</sub> were also examined using only those subjects with TG concentrations <4000 mg/L (n = 313). The PPV of LDL-C estimated by either method decreased as LDL-C concentrations...
increased (PPV range, 91–100% for LDL-C Roche assay and 90–99% for LDL-C Fried; Fig. 4A). In contrast, the NPV of LDL-C estimated by either method increased as LDL-C concentrations increased (NPV range, 80–99% for LDL-C Roche assay and 81–99% for LDL-C Fried; Fig. 4B). The PPVs were similar for both methods, whereas the NPV was slightly better for the LDL-C Fried assay.

POSTPRANDIAL STUDY
As expected, TGs increased significantly (1365 vs 2036 mg/L; \( P < 0.001 \)) after feeding. Statistically significant decreases were also seen in both TC (2009 vs 1973 mg/L; \( P < 0.001 \)) and HDL-C (513 vs 490 mg/L; \( P < 0.001 \)) in samples obtained in the fasting state and postprandially. Although a good correlation was seen in LDL-C Roche concentrations among the paired samples (\( \frac{2009}{2009} = 0.95 \), \( r = 0.98 \)), significant decreases occurred postprandially (2009 vs 1262 mg/L (−6.2%); \( P < 0.001 \)). When we used the LDL-C Fried, which is not recommended in the postprandial state, the equation of the regression line showed a negative intercept (\( \frac{2009}{2009} = 0.97 \), \( r = 0.98 \)), producing a significant discrepancy between the paired samples (1294 vs 1146 mg/L (−11.4%)). However, despite this difference, LDL-C Roche measured after feeding was equally effective in classifying these 43 participants into NCEP cut-points. In fact, 86% of subjects (37 of 43) were correctly classified when nonfasting samples were used compared with 83% (36 of 43) when fasting samples were used.

STORAGE
When serum samples were reanalyzed 12 months after storage at −20 °C, a good agreement (\( \frac{2009}{2009} = 1.012 \), \( r = 0.984 \)) between the LDL-C values and those obtained at baseline was seen in 121 of the 124 samples examined. The three discrepant samples were grossly hypertriglyceridemic (11 590, 16 990, and 16 700 mg/L). After storage, these samples showed a positive bias in the LDL-C Roche assay of 2054, 1189, and 1389 mg/L, respectively. Furthermore, storage of two serum pools at 4 °C for up to 14 days did not affect the measurement of LDL-C by the homogeneous assay (pools 1 and 2, 725 and 1274 mg/L, respectively, at baseline and 730 and 1277 mg/L, respectively, after 14 days).

Discussion
Numerous epidemiological studies and prospective clinical trials have demonstrated that an increased LDL-C concentration is an important independent risk factor for CHD. In addition, the reduction of increased LDL-C is a major goal for the primary and secondary prevention of

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**Table 4. Effect of TG concentration on the classification of subjects correctly into NCEP cut-points using LDL-C Roche and LDL-C Fried.**

<table>
<thead>
<tr>
<th>TGs, mg/L</th>
<th>LDL-C Roche</th>
<th>LDL-C Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt; 2000)</td>
<td>261/306 (85.3%)</td>
<td>271/306 (88.6%)</td>
</tr>
<tr>
<td>2000–3999</td>
<td>88/99 (88.9%)</td>
<td>92/99 (92.9%)</td>
</tr>
<tr>
<td>4000–5999</td>
<td>39/40 (97.5%)</td>
<td></td>
</tr>
<tr>
<td>(\geq 6000)</td>
<td>20/20 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

*a* LDL-C Roche was used as the true value for classification.

*b* Results are given as number of subjects correctly classified/total number of subjects (percentage correctly classified).

*c* The LDL-C Fried was only calculated in samples with TGs \(< 4000\) mg/L.
The Friedewald calculation for estimating the LDL-C concentration is the routine method currently recommended by the NCEP Working Group for Lipoprotein Measurement. Because of the shortcomings of this calculation, methods for the direct determination of LDL-C are needed. Homogeneous methods have the apparent advantage of obviating the need for pretreatment of samples, being performed online, and requiring only a few microliters of sample. The purpose of this multicenter study was to evaluate the analytical and clinical performance of the recently introduced liquid homogeneous LDL-C Roche method.

The NCEP recommends that the total error for LDL-C determination be $<12\%$, which can be achieved by an imprecision of $\leq 4\%$ and a bias from the reference method of $\leq 4\%$ (7). The imprecision criterion was met by all eight laboratories that participated in this study, whereas the bias exceeded $4\%$ at different LDL-C concentrations. This bias tended to increase with decreasing LDL-C concentrations. Because the imprecision seen was low, this homogeneous assay met the NCEP total error requirements in the range of the clinical decision cut-points.

Good agreement was seen between the LDL-C Roche and the LDL-C UC procedures. The bias plots revealed a constant negative bias that was independent of the LDL-C concentration. In samples with TG concentrations $\geq 4000$ mg/L, this negative bias was slightly accentuated when the LDL-C Roche assay was used. However, the negative bias of LDL-C Fried was much more pronounced in such samples, so that this method is obsolete in samples with TG concentrations $\geq 4000$ mg/L, as already published by Friedewald himself (6). In addition, the results of the method comparisons further support that the homogeneous LDL-C Roche assay can be used in the determination of LDL-C in hypertriglyceridemic samples. Therefore, the LDL-C Roche assay provides laboratorians the means to measure LDL-C in samples with increased TGs. The UC method measures the cholesterol component of the wide-density LDL fraction, which includes LDL, intermediate density lipoprotein, and lipoprotein(a). The good agreement between the UC procedure and this homogeneous assay suggests that the latter is capable of measuring the cholesterol content of the broad density LDL fraction. However, the constant negative bias seen between the LDL-C Roche and the UC may be an indication of a suboptimal standardization of the new homogeneous LDL-C Roche assay.

The effects of common interferents were investigated by addition experiments and bias plots using the serum samples included in the method comparison study. Addition experiments showed no significant interference caused by free hemoglobin up to concentrations of at least 6000 mg/L and unconjugated or conjugated bilirubin up to 500 and 250 mg/L, respectively. Different addition experiments with isolated TG-rich lipoproteins showed deviations of $\pm 10\%$ for LDL-C Roche values when TGs exceeded $10000$ mg/L. A slight but incremental negative bias in LDL-C Roche was also apparent from the bias plot as TG concentrations increased. Although this method enables the determination of LDL-C in samples with TG concentrations $>4000$ mg/L, the LDL-C results tended to decrease with increasing TG values, whereas an earlier reported homogeneous method tended to increase (20).

According to the NCEP-ATP II guidelines, the management of hyperlipidemic patients, using either dietary or drug therapy, is based on four LDL-C cut-points (1000, 1300, 1600, or 1900 mg/L). LDL-C concentrations determined by either the LDL-C Roche assay or the adjusted LDL-C Fried correctly classified 86% and 89% of the subjects, respectively, into the above mentioned cut-points.
The LDL-C$_{\text{Roche}}$ assay was able to correctly classify into NCEP cut-points nearly all subjects with TG concentrations $\geq 4000$ mg/L, samples in which the Friedewald LDL should not be applied. This will provide the clinical laboratory the ability to measure LDL-C in hypertriglyceridemic samples and alleviate the need for the expensive, time-consuming, cumbersome UC procedure. Furthermore, our data indicate that the LDL-C$_{\text{Roche}}$ assay is effective in classifying subjects into NCEP cut-points when samples collected postprandially are used.

Our data show that storage at $-20^\circ$C for up to 12 months did not influence the measurement of samples with TG concentrations $<10\ 000$ mg/L. However, in turbid samples with TG concentrations $>10\ 000$ mg/L, unacceptable overestimation of the LDL-C concentration ($>1000$ mg/L) was seen. Therefore, this assay is not adequate for use in grossly lipemic samples stored frozen at $-20^\circ$C. In addition, our data indicate that samples stored at 4 $^\circ$C for up to 2 weeks did not experience any change in their LDL-C concentrations when measured by the homogeneous assay. This observation could have a practical importance to the clinical laboratory.

In conclusion, the homogeneous LDL-C$_{\text{Roche}}$ assay is precise and acceptably accurate. It represents an improvement in the measurement of LDL-C concentration in samples with increased TGs or samples collected postprandially and may assist in the identification of individuals at increased risk of CHD and the management of patients with hyperlipoproteinemia.

We thank Francesca Colella, Philippe Gillery, Michael König, Terence Law, Michael Macke, Winfried März, Claudine Meier, Martin van Vliet, and Heinrich Wieland of the participating laboratories, and Jochen Jarausch, Ann Foster, and Ken Slickers from Roche Diagnostics, all of whom were involved in this study. We also thank Roche Diagnostics for providing the test kits.

References


