Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg$^{2+}$ method

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LDL-cholesterol (LDL-C) concentration is currently determined in most clinical laboratories by the Friedewald calculation. This approach has several limitations and may not meet the current total error requirement in LDL-C measurement of ≤12%. We evaluated the analytical and clinical performance of the direct N-geneous LDL-C assay (Equal Diagnostics). The N-geneous method correlated highly with the modified beta-quantification assay ($r = 0.95$; $y = 0.91x + 70.6$ mg/L; $n = 199$), showed no significant effect of increased triglyceride or other common interferants, and performed adequately in serum samples from nonfasting individuals. This assay demonstrated a mean total error of 6.75% over a wide range of LDL-C concentrations. In addition, at the medical decision cutoff points, this LDL-C assay showed positive predictive values of 78–95% and negative predictive values of 84–99%. We conclude that the N-geneous LDL-C meets the currently established analytical performance goals and appears to have a role in the diagnosis and management of hypercholesterolemic patients.

The diagnosis and management of hypercholesterolemia in adults are largely based on the concentration of LDL-cholesterol (LDL-C). According to the National Cholesterol Education Program (NCEP)-Adult Treatment Panel II (1), LDL-C values $<$1300 mg/L (50th percentile) are considered desirable, and those $>$1600 mg/L (75th percentile) are considered high. Patients with documented coronary heart disease are recommended to maintain their LDL-C concentrations below 1000 mg/L (1).

In most clinical laboratories, LDL-C is currently derived by the Friedewald equation (2). Although the estimation method correlates highly with the beta-quantification, it has several shortcomings (3, 4). This calculation 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 ($>$4000 mg/L). Furthermore, according to the NCEP Laboratory Standardization Panel (LSP), the LDL-C concentration must be determined with a total analytical error not exceeding $\pm 12$% ($\leq\pm 4$% imprecision and $\leq\pm 4$%bias) for a patient to be correctly classified into the NCEP risk categories (4, 5). The Friedewald calculation may not meet these relatively rigid criteria because it combines the inaccuracy and imprecision of three independent measurements: total cholesterol (TC), triglycerides (TGs), and HDL-cholesterol (HDL-C) (3).

Chemical precipitation and immunoseparation methods for the quantification of LDL-C concentration have been reported (4). These methods are affected, to different degrees, by TG concentration, do not measure all “LDL components” as determined by beta-quantification, marginally meet the LSP analytical performance criteria, and require a pretreatment step (6–11). Here we describe the analytical and clinical performance of a new homogeneous method, N-geneous LDL-C (Equal Diagnostics). We also compare this method with the ultracentrifugation/dextran sulfate-Mg$^{2+}$ (UC-DS) method (the modified Lipid Research Clinics beta-quantification method for LDL-C) and the Friedewald calculation.

**Materials and Methods**

**Modified beta-quantification.** A 230-$\mu$L serum sample was centrifuged at 250 000$g$ for 3 h at 10°C in a Beckman TL100 rotor with 7 × 20 mm polyallomer tubes (Beckman...
The tubes were sliced to isolate the triglyceride-rich lipoprotein fractions (density <1.006 kg/L). This separation method has been shown to be comparable with the original beta-quantification method (12). The volume of the infranatant was restored to the original sample by isotonic saline (9 g/L NaCl), and HDL-C was quantified after DS-MgCl₂ precipitation (DS, M₂₆₂ 50 000; concentrations in the specimen, 1 g/L DS and 50 mmol/L MgCl₂). The cholesterol component of LDL is then estimated by subtracting HDL-C from infranatant cholesterol. Our laboratory is involved in the Alert LDL Cholesterol Standardization Program (Pacific Biometrics Research Foundation, Seattle, WA) to periodically check the proficiency of the beta-quantification procedure. Five samples with different LDL-C concentrations were isolated three times a year. The mean ± SD percentage bias for LDL-C concentrations of 620–1770 mg/L for six consecutive surveys was −1.2% ± 2.4%.

TC, TGs, and HDL-C. Serum cholesterol and TG concentrations were determined enzymatically on the Hitachi 911 analyzer (Boehringer Mannheim). The measurement of TGs was corrected for the presence of endogenous glycerol. The HDL fraction was separated, as previously described (13), using DS-MgCl₂ precipitation reagents (same method as described above for the beta-quantification), and the cholesterol component was measured enzymatically on the Hitachi 911 analyzer. TC was determined with a day-to-day variation (CV) of 1.3% at concentrations of 1400 and 2000 mg/L; TG CVs were 2% and 1.6% for concentrations of 1000 and 2000 mg/L, respectively; HDL-C CVs were 2.7% and 2.0% at concentrations of 240 and 480 mg/L, respectively. TC was determined with an average ± SD bias from the Centers for Disease Control and Prevention (CDC) target values of 0.62% ± 1.1% for concentrations ranging from 1278 to 2360 mg/L; the TG average ± SD bias of −0.89% ± 3.1% for concentrations ranging from 536 to 2340 mg/L; and the HDL-C average ± SD bias of −1.0% ± 2.4% for concentrations ranging from 270 to 794 mg/L. Our laboratory is certified by the National Heart, Lung, and Blood Institute and CDC Lipid Standardization Program.

N-geneous LDL-C. This assay was performed according to the manufacturer’s specifications on the Hitachi 911 analyzer. The assay is available from Equal Diagnostics, Exton, PA (cat. no. 7120). After Reagent 1 is mixed with the serum specimen, Detergent 1 specifically disrupts non-LDL lipoproteins and causes the release of cholesterol. The formed free cholesterol, a result of hydrolysis by cholesterol esterase, reacts with cholesterol oxidase, generating hydrogen peroxide. The latter is consumed by a peroxidase in the presence of 4-aminantipyrine to generate a colorless product. On the addition of Reagent 2, Detergent 2 specifically releases cholesterol from LDL particles. A similar enzymatic reaction to that described above occurs, except for hydrogen peroxide, which reacts with N,N’-bis-(4-sulfobutyl)-m-toluidine disodium salt, to generate a colored product. The intensity of the generated color is proportional to the concentration of LDL-C. The assay was calibrated daily, using the calibrators provided (cat. no. 7272; LDL-C concentrations of 0 and 1130 mg/L).

Friedewald calculation. LDL-C was estimated by the Friedewald calculation [LDL-C = TC − (HDL-C + TG/5)], only when fasting TGs are <4000 mg/L, where TG/5 is an estimate of VLDL-cholesterol (VLDL-C), and all concentrations are expressed in mg/L. When values are expressed in mmol/L, VLDL-C is estimated as TG/2.22.

Triglyceride-rich lipoproteins isolation. The triglyceride-rich lipoproteins fraction (>1.006 kg/L) was isolated from serum samples by UC as described above. The isolated fractions of several samples were mixed, and the TG content of the pool was determined.

SAMPLES
One hundred and ninety-nine fasting serum samples were obtained over a 1-month period for the comparison study from the daily pool of received specimens at several clinical chemistry laboratories, including Beth Israel-Deaconess Medical Center, the Veteran Administration Medical Center in Washington, D.C. and Seattle, and Quest Labs.

To determine the postprandial effect on the determination of LDL-C by the N-geneous method, paired 9- to 12-h fasting and nonfasting (3 h after high fat meal) blood samples were obtained from 36 individuals on the same day. The high fat meal consisted of a McDonald’s Sausage McMuffin and hash browns (total fat, 31 g; calories from fat, 180). In addition, to establish the effect of different anticoagulants on the determination of LDL-C by the N-geneous assay, blood samples from 19 individuals were simultaneously collected from a single venipuncture in tubes containing (in random order) EDTA (final concentration 35 g/L), heparin, or no anticoagulant. Serum samples were allowed to sit for 20–30 min at room temperature before centrifugation. EDTA- and heparinized-plasma were separated from blood cells within 15 min of collection.

ANALYTICAL PERFORMANCE EVALUATION
Precision. Within-run and day-to-day precision studies were performed using fresh-frozen human serum with LDL-C concentrations ranging from 832 to 1566 mg/L. Aliquots from the different pools were prepared and stored at −80 °C.

Ascorbate, bilirubin, hemoglobin, and TG interference. Five pools were prepared by combining sera from ~30 individuals. The TC, TG, HDL-C, and LDL-C concentrations of the pools were as follows: pool 1, 1730 mg/L TC, 1190 mg/L TG, 447 mg/L HDL-C, and 910 mg/L LDL-C; pool 2, 1720 mg/L TC, 1670 mg/L TG, 359 mg/L HDL-C, and...
For the ascorbate interference study, various amounts of the stock ascorbic acid solution (1000 mg/L in isotonic saline; Sigma Chemicals) were added to the different pools to give final concentrations of 0, 50, 100, and 150 mg/L. The dilutions were done such that the same amount of saline was delivered to each sample. The concentration of ascorbic acid was confirmed by HPLC (14).

For the bilirubin interference study, various amounts of the stock bilirubin solution (2000 mg/L in 0.1 mol/L sodium hydroxide; Pfanstiehl) were added to the different pools to give final concentrations of 3.4, 41, 108, and 178 mg/L. The dilutions were done such that the same amount of sodium hydroxide was delivered to each sample. The final concentration of total bilirubin was established on the Hitachi 911 with the use of 2,5-dichlorophenylhydrazonium tetrafluoroborate as the diazonium salt, and the absorbance was monitored at 570 nm.

For the hemoglobin interference study, hemolysate was prepared by (a) isolating red blood cells, (b) washing the cells with isotonic saline five times, (c) bursting the cells by freezing, and (d) removing sedimented red cell ghosts and membrane debris by centrifugation at 2000 rpm for 15 min. The hemoglobin concentration in the hemolysate was determined using a Technicon H3™ analyzer. Various amounts of the prepared hemolysate (100 g/L) were added to the different pools to give final hemoglobin concentrations of 0.48, 2.12, 5.20, and 10.30 g/L. The final concentration of hemoglobin was confirmed on the Hitachi 911, using the hemolysis index function.

For the TG interference study, TG-rich lipoprotein fractions were prepared as described above, and various amounts of the isolate (TGs, 45 000 mg/L) were added to the different pools to give mean final TG concentrations of 972, 3084, 6088, and 10 780 mg/L. The concentrations of TGs were determined enzymatically as described above.

**Stability study.** Four serum pools were prepared and stored in small aliquots at either 4, −20, or −80 °C up to 1 month. LDL-C concentrations were measured daily, using the N-geneous assay, over a period of 4 weeks (n = 20).

**Statistical analysis**

The means, medians, and SDs were calculated with Microsoft Excel, Ver. 5.0 (Microsoft). Student's t-test and least-squares linear regression analysis were performed using SigmaPlot statistics program (Jandel Scientific). The t-test was considered significant at P <0.05. Biases were calculated as test procedure result (N-geneous assay or Friedewald calculation) minus reference procedure result.

Total error was calculated as the summation of the systematic and random error [total error (%) = bias + (imprecision x 1.96)] (15, 16). Systematic error, including the constant and the proportional error, was derived from the linear regression equation, \( y = bx + a \), where b was the slope of the regression equation and represented the proportional error and a was the y-axis intercept and represented the constant error. Systematic error at a particular LDL-C concentration (\( x_t \)) was defined as the absolute value of \( y_t - x_t \), where \( y_t = bx_t + a \). Random error was calculated as the day-to-day precision multiplied by 1.96.

The positive predictive value (PPV) of an LDL-C assay at each specified cutoff point was calculated as [true positive/(true positive + false positive)] × 100, where true positive meant that LDL-C results of both the reference procedure and the test method (N-geneous assay or Friedewald calculation) were greater than or equal to the cutoff concentration, and false positive meant that the test method LDL-C result was greater than the cutoff point when the reference procedure LDL-C value was less than the cutoff point. The negative predictive (NPV) value of an LDL-C assay at each specified cutoff point was calculated as [true negative/(true negative + false negative)] × 100, where true negative meant that LDL-C results of both the reference procedure and the test method were less than the cutoff point concentration, and false negative meant that the test method LDL-C result was less than the cutoff point when the reference procedure LDL-C value was greater than or equal the cutoff point concentration. The sensitivity of an assay was calculated as [true positive/(true positive + false negative)] × 100, and the specificity as [true negative/(true negative + false positive)] × 100.

**Results and Discussion**

Lipid and lipoprotein concentration measurements for the Lipid Research Clinics studies were performed using plasma anticoagulated with EDTA. However, clinical laboratories today use only serum or heparinized plasma specimens for their routine lipid testing. Therefore, in this study the effect of the various anticoagulants on the measurement of LDL-C by the N-geneous assay was examined using paired serum, EDTA-treated plasma, and heparinized plasma collected simultaneously from 19 fasting individuals. No significant difference was seen between mean LDL-C concentrations of serum (1057 ± 247 mg/L) and heparinized (1034 ± 218 mg/L) samples (P <0.67). The mean LDL-C value of EDTA-treated plasma (1012 ± 209 mg/L) was ~3% lower, after correction for the osmotic shifting effect of EDTA (values were multiplied by 1.03), than that of serum or heparinized samples. This negative trend, however, was not statistically significant. The exact reason for this negative bias is not known at present. However, it can be attributed either to the possibility of EDTA chelating some important cations (such as Ca²⁺, Mg²⁺, or Fe²⁺) for the optimal performance of the assay or the presence of a higher
concentration of EDTA in the blood collection devices than originally expected, thus requiring correction for fluid shifting of >3% (17, 18). The latter explanation appears more likely because a persistent negative bias of 2–3% was also noted in measured TGs and TC.

The reproducibility of the assay was examined using fresh-frozen serum pools with different LDL-C concentrations. The within-run imprecision values (n = 20) at LDL-C concentrations of 832, 1156, and 1566 mg/L were 1.63%, 1.71%, and 2.68%, respectively. Furthermore, the day-to-day imprecision values (n = 21) at LDL-C concentrations of 900, 965, 1060, and 1291 mg/L were 3.1%, 3.2%, 3.2%, and 3.1%, respectively. According to the NCEP-LSP performance goals, LDL-C must be determined with an imprecision of <±4%. The N-geneous assay has met this performance criterion in serum pools with different LDL-C concentrations.

For any new assay to be accepted clinically, it must demonstrate comparable performance to the beta-quantification method, which is the accuracy base on which the NCEP cutoff points are determined. The LDL-C concentrations of the serum samples collected from fasting normolipidemic and hyperlipidemic individuals were simultaneously determined, over a 1-month period and 15 different analytical runs, by the modified beta-quantification method (UC-DS), the N-geneous assay, and the currently recommended routine method, the Friedewald calculation. The LDL-C concentrations were calculated by the latter method only for those samples with TG concentrations <4000 mg/L. The lipid and lipoprotein concentrations of the study population are presented in Table 1. The comparison-of-methods plot [UC-DS (y) vs test method (y)] showed a least-squares linear regression equation of y = 0.91x + 70.6 mg/L (n = 199; r = 0.95) for the N-geneous assay and y = 0.96x + 43.4 mg/L (n = 164; r = 0.94) for the Friedewald calculation (Fig. 1). To better illustrate the performance of the N-geneous assay in hypertriglyceridemic samples, specimens with TGs <4000 mg/L and those with TGs ≥4000 mg/L were compared separately with the UC-DS assay. Least-squares linear regression equations of y = 0.89x + 89.8 mg/L (n = 164; r = 0.94) for samples with TGs <4000 mg/L and y = 1.02x – 29 mg/L (n = 35; r = 0.96) for samples with TGs ≥4000 mg/L were obtained. In addition, Table 1 indicates a mean LDL-C bias ± SD, as determined by the N-geneous assay of –48.4 ± 119 mg/L for the total population (n = 199), –58.4 ± 118 mg/L for those with TGs <4000 mg/L (n = 164), and –1.6 ± 110 mg/L for those with TGs ≥4000 mg/L (n = 35). This study demonstrates that LDL-C measured by the N-geneous assay is comparable with that determined by the modified beta-quantification method. Our findings also suggest that this method measures the cholesterol of the "LDL components", as determined by beta-quantification, which includes LDL, intermediate-density lipoproteins, and lipoprotein(a). However, the exact reason for the negative bias seen in samples with TGs <4000 mg/L is not known at the present. Perhaps the cholesterol component of either LDL, intermediate-density lipoproteins, or lipoprotein(a) is not totally measured by the N-geneous assay in the absence of increased TGs. This observation could represent a limitation of the N-geneous assay because the great majority of the population has TGs <4000 mg/L.

No significant bias from LDL-C measured by the reference procedure was seen over a wide range of TG concentrations (370–11 320 mg/L; Fig. 2A), and the addition of various amounts of TG-rich lipoproteins (up to 10 780 mg/L) to five different pools revealed no statistically significant change in measured mean LDL-C values by the N-geneous assay. The mean and SD of LDL-C concentrations at baseline were 699 ± 181 mg/L (mean TGs, 972 mg/L); after the addition of TG-rich lipoproteins, the mean and SD were 696 ± 177, 694 ± 173, and 685 ± 176 mg/L at mean TG concentrations of 3084, 6088, and 10 780 mg/L, respectively. The experiments clearly indicate that, unlike previously described methods (6–11), the N-geneous assay for LDL-C is not affected by the presence of increased concentrations of TG. In addition, no significant bias in calculated LDL-C by the Friedewald equation from the reference procedure (Fig. 2B) was seen with increasing TG concentrations up to 4000 mg/L.

The NCEP performance goals require that LDL-C be

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**Table 1. Characteristics of the groups used in this study.**

<table>
<thead>
<tr>
<th>Total sample number</th>
<th>Total population</th>
<th>TG &lt;4000 mg/L</th>
<th>TG ≥4000 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample number</td>
<td>199</td>
<td>164</td>
<td>35</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2164 ± 460</td>
<td>2092 ± 409</td>
<td>2480 ± 560</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2181 ± 1951</td>
<td>1425 ± 872</td>
<td>5727 ± 1819</td>
</tr>
<tr>
<td>Median</td>
<td>1420</td>
<td>1200</td>
<td>5230</td>
</tr>
<tr>
<td>Range</td>
<td>370–11 320</td>
<td>370–3930</td>
<td>4000–11 320</td>
</tr>
<tr>
<td>HDL-C</td>
<td>481 ± 170</td>
<td>517 ± 164</td>
<td>308 ± 54</td>
</tr>
<tr>
<td>UC-DS method, LDL-C</td>
<td>1255 ± 378</td>
<td>1302 ± 359</td>
<td>1035 ± 424</td>
</tr>
<tr>
<td>Friedewald, LDL-C</td>
<td>1295 ± 365</td>
<td>1295 ± 365</td>
<td></td>
</tr>
<tr>
<td>Bias</td>
<td>–7.0 ± 121</td>
<td>–7.0 ± 121</td>
<td></td>
</tr>
<tr>
<td>N-geneous assay, LDL-C</td>
<td>1206 ± 361</td>
<td>1243 ± 337</td>
<td>1033 ± 424</td>
</tr>
<tr>
<td>Bias</td>
<td>–48.4 ± 119</td>
<td>–58.4 ± 118</td>
<td>–1.6 ± 110</td>
</tr>
</tbody>
</table>

*All concentrations are expressed in mg/L; results are presented as mean ± SD, except when noted.
measured with an analytical bias of $\leq 4\%$ (5). The substitution of LDL-C values used for medical decisions ($1000–1900\, \text{mg/L}$) into the least-squares linear regression equation for the N-geneous assay yielded systematic errors that ranged from 0.4% to 1.6%. According to the NCEP-LSP, the total allowable analytical error, which is the combination of random error and systematic error, for LDL-C must not exceed $\pm 12\%$ (5). When calculated at the different LDL-C concentrations used in the day-to-day precision study (900, 965, 1060, and 1291 mg/L), the average total analytical error of the N-geneous assay was
Therefore, the N-geneous assay appears to analytically meet the performance goals established by the NCEP. For the sake of comparison, the same parameters were estimated for the Friedewald calculation. The bias and imprecision values for TC, HDL-C, and TGs were combined. The average systematic error, random error (imprecision multiply by 1.96), and total error for the calculated LDL-C were 0.8%, 10.8%, and 11.6%, respectively.

Bilirubin and ascorbic acid are reducing compounds.
that are known to interfere with peroxidase-dependent cholesterol measurement. In addition, hemoglobin is a common interferant in routine clinical laboratory testing. In this study, the potential interference from these three analytes was evaluated by the addition of increasing and known concentrations of hemoglobin, bilirubin, or ascorbate to different serum pools (Fig. 3). A slight positive trend was observed in LDL-C values as hemoglobin concentration increased in the test pools (Fig. 3A). These differences, however, fell short of statistical significance. In contrast, a slight negative trend was observed in LDL-C values as bilirubin concentrations increased in the various pools (Fig. 3B). The addition of up to 258 mg/L bilirubin caused LDL-C concentrations to decrease by no more than 5% (data not shown). This difference was not statistically significant. Concurrently, the addition of up to 150 mg/L of ascorbic acid did not result in a significant change in LDL-C values as bilirubin concentrations increased in the various pools as measured by the N-geneous assay (Fig. 3C). Our findings indicate the N-geneous assay is apparently unaffected by these common interferants.

In this study, the effect of feeding on the determination of LDL-C concentration by the N-geneous assay was examined in paired samples from 36 subjects. As expected, TGs increased significantly after feeding (1090 ± 635 mg/L vs 1830 ± 1090 mg/L; P < 0.0001). However, no statistically significant difference was seen in TC [fasting (mean ± SD) 1943 ± 385 mg/L vs nonfasting 1952 ± 368 mg/L], HDL-C (560 ± 156 mg/L vs 550 ± 167 mg/L) or LDL-C (1122 ± 299 mg/L vs 1113 ± 273 mg/L) concentration in samples obtained in the fasting state and postprandially. It is important to note, however, that 2 of the 36 subjects who participated in this study would have been misclassified into NCEP risk categories if postprandial serum samples were used in the determination of their LDL-C (subject 1, 1580 mg/L fasting vs 1200 mg/L nonfasting; subject 2, 1480 mg/L fasting vs 1120 mg/L nonfasting). Parallel decreases in TC postprandially have also been noted in the same two subjects and would have caused them to be misclassified into NCEP risk categories as well (subject 1, 2530 mg/L fasting vs 2000 mg/L nonfasting; subject 2, 2340 mg/L fasting vs 1920 mg/L nonfasting). Compared with values in the fasting state, LDL-C determined postprandially had a PPV of 100% and an NPV of 93%. This assay appears to perform better than the LDL-C immunoprecipitation method in postprandial samples (7). To further characterize the performance of the assay, the fasting and nonfasting LDL-C values were examined separately in the 11 hypercholesterolemic (LDL-C >1300 mg/L) and the 25 normocholesterolemic subjects in this group. In the hypercholesterolemic group, the fasting and nonfasting mean ± SD of LDL-C concentrations were 1475 ± 117 mg/L and 1404 ± 142 mg/L (P < 0.23), respectively. The two discrepant subjects described above fell into this category. In the normocholesterolemic group, the fasting and nonfasting LDL-C values were 967 ± 207 mg/L and 984 ± 211 mg/L (P < 0.78), respectively. Physiological changes in lipoprotein concentrations are known to occur postprandially. An average decrease of 22% in LDL-C concentration as determined by beta-quantification 3 h after fat feeding has been reported (19). It is not known at present why the N-geneous LDL-C assay is unaffected postprandially. Perhaps some VLDL remnants, which are increased postfeeding, are measured as part of LDL-C, thus compensating for the physiologically decreased LDL.

A stability study was conducted to determine a possible change in LDL-C concentrations of four different freshly prepared serum pools stored at 4, −20, or −80 °C for up to 4 weeks. The mean (SD) of LDL-C concentrations of the various pools at baseline and after 1-week, 2-week, 3-week, and 4-week storage at 4°C, −20°C, and −80°C are shown in Table 2. The effect of short-term storage on the measurement of LDL-C concentration by the N-geneous assay in four different serum pools.

### Table 2. The effect of short-term storage on the measurement of LDL-C concentration by the N-geneous assay in four different serum pools.

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Baseline</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 °C</td>
<td>1053 (180)</td>
<td>1038 (157)</td>
<td>1085 (166)</td>
<td>1053 (180)</td>
<td>1058 (177)</td>
</tr>
<tr>
<td>−20 °C</td>
<td>1050 (177)</td>
<td>1043 (187)</td>
<td>1070 (194)</td>
<td>1078 (187)</td>
<td>1073 (182)</td>
</tr>
<tr>
<td>−80 °C</td>
<td>1040 (154)</td>
<td>1028 (189)</td>
<td>1055 (162)</td>
<td>1065 (174)</td>
<td>1068 (176)</td>
</tr>
</tbody>
</table>

* Values are expressed in mg/L.

### Table 3. Reliability of various screening strategies in detecting subjects with hypercholesterolemia (LDL-C ≥ 1300 mg/L as determined by the modified beta-quantification method).

<table>
<thead>
<tr>
<th>Screening strategy</th>
<th>Sensitivity a</th>
<th>Specificity a</th>
<th>PPV a</th>
<th>NPV a</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (≥2000 mg/L)</td>
<td>98</td>
<td>60</td>
<td>65</td>
<td>97</td>
</tr>
<tr>
<td>TC - (HDL-C + 300 mg/L) (≥1300 mg/L)</td>
<td>87</td>
<td>71</td>
<td>70</td>
<td>88</td>
</tr>
<tr>
<td>Friedewald LDL-C (≥1300 mg/L)</td>
<td>91</td>
<td>90</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>N-geneous LDL-C (≥1300 mg/L)</td>
<td>77</td>
<td>92</td>
<td>88</td>
<td>84</td>
</tr>
<tr>
<td>N-geneous LDL-C (≥1200 mg/L)</td>
<td>91</td>
<td>81</td>
<td>79</td>
<td>91</td>
</tr>
</tbody>
</table>

* All values are presented as percentages.
* Only subjects with concentrations <4000 mg/L are included.
3-week, and 4-week storage periods at -4, -20, or -80 °C are presented in Table 2. None of these changes were statistically significant. The short-term stability of this assay at various temperatures provides the clinical laboratory with an added practical advantage and suggests a role for this method in clinical and epidemiological studies. However, a long-term stability study must demonstrate the lack of effect from storage at -20 or -80 °C on LDL-C values before the N-geneous method is considered for longitudinal trials. Such a study is ongoing in our laboratory at present.

Total cholesterol has been used as a surrogate test for LDL-C in screening for hypercholesterolemia. We evaluated the N-geneous LDL-C assay as a potential screening test for high cholesterol in this study population (n = 199). Hypercholesterolemia was defined as LDL-C ≥1300 mg/L, as determined by beta-quantification. The sensitivity and specificity of TC (at concentrations ≥2000 mg/L) as the screening test were 98% and 60%, respectively (Table 3). The high incidence of false positives when TC is used usually diminishes by the measurement of HDL-C along with TC at the initial screening stage, as has been recommended by the NCEP-Adult Treatment Panel II (1). In this study population, when TC – (HDL-C + 300 mg/L), which represents the mean VLDL-C in American adults) was used (at concentrations ≥1300 mg/L), the specificity was increased by over 10% (Table 3). The sensitivity and specificity of LDL-C (at concentrations ≥1300 mg/L), as determined by the N-geneous assay, were 77% and 92%, respectively. The sensitivity of the N-geneous LDL-C assay, however, improved when a lower LDL-C cutoff point of ≥1200 mg/L was used to detect hypercholesterolemic subjects (LDL-C ≥1300 mg/L by beta-quantification; Table 3). Our data indicate that the direct measurement of LDL-C by this assay, using a lower cutoff point, has better specificity (81% vs 71%) and sensitivity (91% vs 87%) than the TC – (HDL-C + 300 mg/L) currently used in screening for hypercholesterolemia. Furthermore, we evaluated the suitability of the N-geneous LDL-C method in the management of patients with hypercholesterolemia. According to the NCEP-Adult Treatment Panel II guidelines (1), the management of hyperlipidemic patients, using either dietary or drug therapy, is based on four LDL-C cutoff points (≥1000, ≥1300, ≥1600, or ≥1900 mg/L). The ability of this assay to correctly classify subjects at the medical decision cutoff points was evaluated in this study population (n = 199). For the interest of comparison, the PPV and NPV of LDL-C estimated by the Friedewald calculation were also examined, using only those subjects with TGs <4000 mg/L (n = 164). LDL-C concentrations determined by either the Friedewald calculation or the N-geneous assay classified over 80% of the subjects into the above mentioned cutoff points, correctly. The PPV of LDL-C estimated by either method decreased as LDL-C concentrations increased (PPV range 95–78% for N-geneous assay and Friedewald calculation; Fig. 4A). In contrast, the NPV
of LDL-C estimated by either method increased as LDL-C concentrations increased (NPV range 84–99% for N-geneous assay and 88–99% for Friedewald calculation; Fig. 4B). About 65% of patients with LDL-C ranging from 1300 to 1590 mg/L were classified into this category by either the N-geneous assay or the Friedewald calculation (N-geneous PPV, 64%; NPV, 84%; Friedewald PPV, 69%; NPV, 89%). The N-geneous assay appears to be capable of classifying patients into NCEP cutoff points, regardless of their TG concentrations.

In conclusion, the N-geneous LDL-C assay meets the established NCEP analytical performance goals, is unaffected by major interferants, including increased TGs, and appears to be a potential screening test for hypercholesterolemia and a possible useful tool in the management of hyperlipidemic patients.

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References