Comparison of two assays for measuring LDL cholesterol

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The purpose of this study was to evaluate the LipiDirect assay (L-LDL) against the Direct LDL immunoseparation method (D-LDL) and beta quantification (BQ-LDL) for measurement of LDL cholesterol (LDL-C) in patients with normo- and hypertriglyceridemia. Samples from 156 patients [triglyceride (Tg) range 0.61–9.95 g/L] were assayed for LDL-C concentrations with the three methods. An additional seven patients with type III hyperlipidemia and 25 paired sera from fasting and nonfasting individuals also were analyzed by the three methods. Both assays displayed excellent precision. The mean LDL-C value from L-LDL was significantly higher than BQ-LDL and D-LDL in normo- and hypertriglyceridemic samples ($P < 0.001$). The mean absolute bias of L-LDL vs BQ-LDL was 12.7% for Tg < 4 g/L and 30.6% for Tg ≥4 g/L, compared with 6.2% and 12.5%, respectively, for D-LDL. L-LDL correctly classified only 68% of patients with LDL-C <1.30 g/L and 57% of patients with LDL-C between 1.30–1.59 g/L as compared with 98% and 93%, respectively, for D-LDL ($P < 0.001$). In patients with type III hyperlipidemia, L-LDL had a 130% positive bias with BQ-LDL as compared with a 14% negative bias for D-LDL. With all three methods there were no significant differences between samples from fasting and nonfasting individuals. On the basis of these findings, the D-LDL assay appears to be superior to the L-LDL assay.

INDEXING TERMS: coronary artery disease • beta quantification • hypertriglyceridemia • immunoseparation

The association between increased concentrations of LDL cholesterol (LDL-C) and increased risk of premature coronary artery disease (CAD) has been clearly demonstrated [1–3]. Intervention with both dietary or drug therapy to lower LDL-C has been shown to decrease both cardiovascular morbidity and mortality [4–6]. Because of this strong and positive association between LDL-C and CAD, the Adult Treatment Panel of the National Cholesterol Education Program (NCEP) as well as the NCEP’s Children and Adolescents Treatment Panel have made concentrations of LDL-C the primary basis for classification and treatment of hyperlipidemia [6].

Given the importance of LDL-C in the diagnosis, classification, and subsequent management protocols for hyperlipidemias, there exists a need for reliable methodologies for determining concentrations of LDL-C in serum for routine use in the clinical laboratory. However, as yet, there is no consensus-approved and consensus-validated “user friendly” method for measuring LDL-C as exists for total cholesterol. Most laboratories use indirect methods for the measurement of LDL-C. The currently accepted “gold standard,” beta quantification (BQ-LDL), involves ultracentrifugation of the serum sample for 18 h [7]. This is a cumbersome procedure, and is labor intensive and technique dependent. It requires expensive instrumentation that preempts its extension to the routine laboratory. In spite of being the reference method, it still involves an indirect measurement of serum LDL-C.

The Friedewald formula is still used by most laboratories to estimate LDL-C concentrations [8]. The equation has been clearly shown to be invalid in hypertriglyceridemic patients [triglycerides (Tg) ≥4 g/L] and in type III hyperlipidemias [9–12]. Also, a fasting sample is required to avoid a Tg bias. In light of this, Genzyme Diagnostics developed an assay for the direct measurement of LDL-C (available from Sigma Diagnostics), the Direct LDL™ (D-LDL). It is an immunoseparation method that includes antibodies against apolipoprotein (apo) AI and apo E to

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1 Nonstandard abbreviations: LDL-, HDL-C, low-density-lipoprotein, high-density lipoprotein cholesterol; CAD, coronary artery disease; NCEP, National Cholesterol Education Program; Tg, triglycerides; apo, apolipoprotein; BQ-LDL, beta quantification LDL-C; L-LDL, LipiDirect LDL-C; D-LDL, Direct LDL immunoseparation cholesterol assay; ICC, intraclass correlation coefficient; and TRL, triglyceride-rich lipoproteins.
remove HDL and VLDL fractions, respectively, allowing LDL-C to be directly measured in the filtrate. Studies have demonstrated that this method has a good correlation with BQ-LDL for measurement of LDL-C in normo- and hypertriglyceridemic patients as well as in type III hyperlipidemic patients [10, 13, 14]. The method is rapid, cost effective, and suitable for routine testing. Recently, Polymedco introduced another method for the measurement of LDL-C called LipiDirect (L-LDL). This method involves a buffered heparin reagent to precipitate LDL, leaving HDL and VLDL in the supernatant. LDL-C is then obtained indirectly by subtracting the cholesterol concentration of the supernatant from the total cholesterol. This method claims to have the same advantages of being rapid, cost effective, and suited for the routine laboratory.

Since there appears to be no published reports comparing the new L-LDL assay with BQ-LDL or the D-LDL immunoseparation assay, the present study was undertaken to evaluate the validity of L-LDL against BQ-LDL (reference method) as well as compare it with the D-LDL immunoseparation assay in patients with normo- and hypertriglyceridemia and in type III hyperlipidemia.

**Materials and Methods**

**REAGENTS**

L-LDL precipitating reagent and controls were obtained from Polymedco (Cortlandt Manor, NY); D-LDL cholesterol kit, cholesterol reagent, and cholesterol calibrator were from Sigma Diagnostics (St. Louis, MO); serum separation tubes were from Becton Dickinson (Franklin Lakes, NJ); Paramax cholesterol and Tg reagent tablets and HDL-C precipitating reagents were from Dade International (Miami, FL); Magnetic HDL-C reagent (Polymedco) and heparin–manganese were from Dade International.

**PATIENTS**

Serum and potassium EDTA plasma samples obtained from 156 fasting (12-h) patients at the Lipid Clinic at Parkland Memorial Hospital were tested for LDL-C concentrations by three different methods—L-LDL, D-LDL, and BQ-LDL. Each sample was analyzed by all three assays within the same week. Since the Friedewald equation is not valid for samples with Tg concentrations ≥4 g/L, this concentration was defined as “hypertriglyceridemia” for the purpose of this study. Of the 156 patients, 106 had Tg <4 g/L (0.61–3.96 g/L) and the remaining 50 patients had Tg concentrations ≥4 g/L (4.00–9.95 g/L). Patients with fasting Tg concentrations >10 g/L were excluded from the study because the L-LDL package insert did not recommend the assay in patients with fasting Tg above this concentration. In addition, seven patients with type III hyperlipidemia were also evaluated by the three methods (type III hyperlipidemia was diagnosed both clinically and if there was a combined hyperlipidemia and VLDL-C/total Tg ratio was ≥0.30) [15]. To determine the effect of the postprandial state on the L-LDL assay, paired fasting and nonfasting samples from 25 subjects were evaluated for LDL-C by all three methods.

**PROCEDURES**

**Quantification of lipids.** Total Tg and cholesterol were measured enzymatically on the Paramax Rx (unless otherwise indicated) with the appropriate reagents from Dade International as described previously [10]. Any sample not analyzed the same day was refrigerated at 4 °C. This laboratory is accredited by the College of American Pathologists and participates in the ALERT proficiency program (Pacific Biometrics) for lipoprotein analysis.

**L-LDL assay.** The assay was performed as per the manufacturer’s protocol. Serum samples and controls measuring 50 µL were added to microcentrifuge tubes containing 400 µL of LDL precipitating reagent. After immediate vortex-mixing, the specimens were allowed to incubate for 20 min at room temperature. The tubes were then centrifuged for 10 min at 2000g and the clear supernatant was transferred to a separate tube. The cholesterol content in the supernatant was determined on the Cobas Mira with the cholesterol reagent and calibrator from Sigma. LDL-C was determined by subtracting the supernatant cholesterol from the total serum cholesterol.

**D-LDL immunoseparation assay.** The assay was performed as previously described [10]. Briefly, LDL-C reagent (200 µL) was pipetted into separation tubes provided in the kit. Controls and serum samples (30 µL) were added to the tubes containing latex beads. After immediate vortex-mixing, the tubes were incubated for 10 min and centrifuged at 6000g for 5 min. The cholesterol in the filtrate was measured on the Cobas Mira with the cholesterol reagent and calibrator from Sigma. Cholesterol calibration was closely monitored with the ALERT standardization program for LDL-C. An average bias of 3.6% was attained with the ALERT proficiency program for D-LDL-C.

**BQ-LDL by ultracentrifugation.** Two milliliters of plasma at a density of 1006 g/L was centrifuged at 4 °C in a fixed-angle rotor for 18 h at 109,000g [10]. The top and bottom fractions were collected into separate tubes and the bottom fraction was brought up to 2 mL with saline. Cholesterol was determined in the plasma and the two fractions. Percent recoveries, determined from the sum of the cholesterol in the two fractions and compared with the total plasma cholesterol, were within 100.2% ± 2.32%. LDL-C from BQ-LDL was obtained by subtracting HDL-C (obtained by precipitation) from the bottom fraction cholesterol. An average bias of 3.5% was attained with the ALERT proficiency program for LDL-C by BQ-LDL.

**Precision studies.** For the L-LDL intraassay precision tests, three serum samples with LDL-C concentrations of 0.84,
1.61, and 2.26 g/L were each assayed 20 times with a single reagent lot. For the interassay precision tests, three samples with 0.77, 1.52, and 2.10 g/L LDL-C were assayed in duplicate for 8 days over a 10-day period. The precision studies for the D-LDL immunoseparation assay and BQ-LDL have been reported previously [10].

Fasting and postprandial samples. To determine the effect of the postprandial state on LDL-C measurement by L-LDL, 25 healthy subjects were asked to fast overnight for at least 12 h. Their blood was drawn in the morning by venipuncture into EDTA and serum separator tubes. The subjects were requested to consume a standard high-fat meal (available from an in-house fast-food outlet and containing 37 g of fat and 0.26 g of cholesterol); a second blood sample was drawn 3.5 h later. Fasting and postprandial samples were then assayed for LDL-C by the three methods.

Statistical analysis. Linear regression analysis was used to compare LDL-C values obtained by the L-LDL assay and D-LDL method against the reference method (BQ-LDL) in normo- and hypertriglyceridemic (Tg ≥ 4 g/L) samples. The Wilcoxon test was used to compare data that were not normally distributed, whereas the paired t-test was used for data that was normally distributed. The mean absolute bias \( \Sigma (|x_i - \bar{x}|)/n \) was calculated for L-LDL and D-LDL methods compared with BQ-LDL. The intraclass correlation coefficient (ICC) was used in evaluating the performance of L-LDL against D-LDL in type III hyperlipidemic patients. The McNemar test was used to compare appropriate classification of LDL-C at the NCEP cutoff values of 1.30 and 1.60 g/L for L-LDL and D-LDL.

Results

The precision profile previously reported for the intra- and interassay CVs for D-LDL was <3.3% and <4.5%, respectively, for LDL-C concentrations ranging from 0.90 to 2.26 g/L; the intraassay CV of BQ-LDL in the same study was <3.6% [10]. Preliminary precision profiles performed during the present study revealed similar findings. The precision profile for L-LDL assay was performed with normal, borderline, and high concentrations of LDL-C (range 0.68–2.31 g/L). The intraassay and interassay CVs of L-LDL for all three concentrations of LDL-C were <3% and <8%, respectively (Table 1).

Comparison of LDL-C values obtained from L-LDL, D-LDL, and BQ-LDL

Linear regression analysis was performed for L-LDL and D-LDL vs BQ-LDL for normo- and hypertriglyceridemic subgroups. As seen in Fig. 1a and 1b, both L-LDL and D-LDL show good correlation with BQ-LDL for Tg concentrations <4 g/L (r = 0.95 and 0.97, respectively). Fig. 2a and 2b shows the good correlation of L-LDL and D-LDL with BQ-LDL for Tg concentrations ≥4 g/L (r = 0.91 and 0.94, respectively). For the comparison studies, LDL-C concentrations were measured in 156 samples (Tg range 0.61–9.95 g/L) by the three assays. Table 2 shows the mean ± SD LDL-C obtained by each method for the overall Tg range as well as in normo- (<4 g/L) and hypertriglyceridemic (≥4 g/L) samples. The mean LDL-C value of L-LDL was significantly higher when compared with D-LDL as well as BQ-LDL for the overall Tg range.

![Fig. 1. Linear regression analysis plot of L-LDL vs BQ-LDL (a) and D-LDL vs BQ-LDL (b) for Tg <4 g/L.](image-url)
and in both normo- and hypertriglyceridemic subgroups \((P < 0.001)\). The mean LDL-C value of D-LDL did not show any statistical difference with BQ-LDL for the overall Tg range and in samples with Tg < 4 g/L but was significantly higher than BQ-LDL in samples with Tg \(\geq 4\) g/L \((P < 0.001)\).

Bias plots of L-LDL and D-LDL against BQ-LDL concentrations and increasing Tg concentrations are shown in Figs. 3 and 4, respectively. As seen in Fig. 3, there is a mean absolute bias of 0.22 g/L for L-LDL, whereas the mean absolute bias for D-LDL is only 0.10 g/L. A larger positive bias is seen with L-LDL than with D-LDL in Fig. 4, which becomes more pronounced in hypertriglyceridemic samples (including some biases >0.50 g/L for L-LDL). The mean absolute percentage bias of L-LDL vs BQ-LDL was 18.5% for the entire range of Tg values, 12.7% for Tg < 4 g/L, and 30.6% for Tg \(\geq 4\) g/L, whereas the mean absolute bias for D-LDL vs BQ-LDL was 8.3% for the entire range of Tg values, 6.2% for Tg < 4 g/L, and 12.5% for Tg \(\geq 4\) g/L.

**APPROPRIATE CLASSIFICATION OF PATIENTS BY L-LDL AND D-LDL ASSAYS WITH THE NCEP LDL-C CUTOFF POINTS**

The NCEP has laid down LDL-C values of <1.30, 1.30–1.59, and \(\geq 160\) g/L as cutoff points for classifying patients without CAD with normal, borderline, and high LDL-C. Since these cutoffs are crucial in therapeutic decision making, we studied the appropriate classification by L-LDL and D-LDL assays into the three categories with LDL-C values coinciding within \(\pm 10\%\) of BQ-LDL (reference cholesterol value).

As seen in Table 3, with BQ-LDL as the point of reference, L-LDL correctly classified only 68% of patients with LDL-C < 1.30 g/L, whereas D-LDL values coincided within \(\pm 10\%\) of BQ-LDL in 98% of these patients. Similarly for concentrations 1.30–1.59 g/L, L-LDL and D-LDL correctly classified 57% and 93% of the patients, respectively. The McNemar test was used to compare the appropriate classification of the two methods for patients with LDL-C < 1.30 g/L \((P = 0.0001)\) and with LDL-C 1.30–1.59 g/L \((P = 0.0006)\). For LDL-C values > 1.60 g/L, the appropriate classification by both assays was 100%.

**COMPARISON OF MEAN LDL-C DETERMINED BY L-LDL, D-LDL, AND BQ-LDL ASSAYS IN TYPE III HYPERLIPIDEMICS**

Because the Friedewald equation is invalid in type III hyperlipidemia, having an assay that can reliably determine LDL-C concentrations in these patients is essential. To test the validity of the L-LDL test in type III hyperlipidemia, LDL-C was determined in seven patients by all three methods. As seen in Table 4, the median LDL-C determined by L-LDL was significantly higher than BQ-LDL (2.72 vs 1.03 g/L), with a mean positive bias of 130%. In contrast, D-LDL showed a mean negative bias of 14% from the BQ-LDL. We also compared the mean LDL-C values for L-LDL and D-LDL with BQ-LDL by using an ICC to express and compare the reliability index of the test result between the two assays. The ICC for L-LDL was 0.82 in type III hyperlipidemics as compared with 0.99 for D-LDL, clearly demonstrating the superiority of the latter assay.

| Table 2. Comparison of LDL-C determined by L-LDL and D-LDL assays with BQ-LDL. |
| Mean ± SD, g/L |
|------------------|------------------|------------------|
| Overall          | 156              | 1.49 ± 0.46a,b   | 1.30 ± 0.42       | 1.29 ± 0.41 |
| Tg < 4 g/L       | 106              | 1.46 ± 0.47a,b   | 1.29 ± 0.44       | 1.32 ± 0.41 |
| Tg ≥ 4 g/L       | 50               | 1.56 ± 0.44a,b   | 1.32 ± 0.44a      | 1.23 ± 0.41 |

\(a\) Significantly different from BQ-LDL by Wilcoxon, \(P < 0.001\).

\(b\) Significantly different from D-LDL by Wilcoxon, \(P < 0.001\).
The validity of l-LDL and d-LDL assays in the postprandial state

Table 5 shows the effect of postprandial state on the concentrations of total cholesterol, Tg, and LDL-C (as measured by the three methods) in 25 subjects. Total cholesterol showed no statistical difference between fasting and nonfasting samples, whereas the Tg was significantly higher in postprandial samples. The results for L-LDL, D-LDL, and BQ-LDL were not significantly different in the fasting and nonfasting states.

Discussion

LDL-C is a key factor in the pathogenesis of premature CAD [1–3]. Assessment of total cholesterol concentrations only to determine patients at risk can be misleading in that an increased HDL-C can result in mild hypercholesterolemia. Clearly, then, the availability of an accurate and precise method to evaluate LDL-C is of key importance in the clinical assessment of patients at risk for CAD.

Currently, most routine laboratories continue to use the Friedewald equation to calculate the concentrations of serum LDL-C [8]. The traditional approach has been to use the equation only in fasting patients with Tg < 4 g/L because the results are unreliable under the following circumstances: (a) when chylomicrons are present; (b) when plasma Tg exceeds 4 g/L; and (c) in patients with type III hyperlipidemia [9–12, 16, 17]. This occurs because the equation is based on the assumption that the majority of circulating Tg resides in the VLDL fraction and that the relation between Tg and cholesterol in this fraction is constant. Particles found in hypertriglyceridemic samples that are generally called “VLDL” are often, in fact, a heterogenous mix of VLDL and chylomicron remnants. It would be more precise to call such particles Tg-rich lipoproteins (TRL) [13]. The percentage of cholesterol is very different across this range of particles and as the Tg concentration rises, fewer of them may contain the usual 20% cholesterol. Errors in calculating the TRL cholesterol will ultimately reflect as an error in LDL-C. Type III hyperlipidemics have increased concentrations of IDL and VLDL remnants in their serum. This fraction (beta VLDL on electrophoresis) contains proportionately more cholesterol than normal VLDL and use of the Friedewald equation in these patients clearly leads to inaccurate LDL-C values [15]. The NCEP recommendation states that in the above circumstances, LDL-C should be measured with the combined ultracentrifugation–polyanion precipitation method (BQ-LDL). Unfortunately, this method is both time consuming and labor intensive, and requires ultracentrifugation that is not available for routine use in most laboratories. In spite of being a reference method, it still calculates LDL-C indirectly. Also, what we commonly refer to as “LDL-C” by BQ-LDL actually represents cholesterol contained in LDL plus IDL plus lipoprotein(a).
and the measurement might better be considered to represent the cholesterol contained in several potentially atherogenic particles [7, 16].

In the search for an assay for the direct quantification of LDL-C, Genzyme Diagnostics (Cambridge, MA) developed an immunoseparation method (available through Sigma Diagnostics)—the D-LDL assay—in 1994. Jialal et al. [10], Pisani et al. [13], and McNamara et al. [14] have all compared this immunoseparation method for direct LDL-C measurement with BQ-LDL and found good correlation between the two methods for all Tg concentrations. No significant differences were found in LDL-C concentrations between the D-LDL assay and BQ-LDL in patients with type III hyperlipidemia in the study by Jialal et al. [10]. The L-LDL assay marketed by Polymedco claims to be another simple, rapid, and cost-effective test for measuring LDL-C. The NCEP has laid down clear analytical goals for the acceptibility of any new assay measuring LDL-C [17]. Previous studies have clearly shown D-LDL to have precision values that fall within these guidelines [10, 13, 14]. For L-LDL, the intraassay

Table 3. Appropriate classification of LDL-C by the L-LDL and D-LDL assays compared with BQ-LDL at the NCEP cutpoints of 1.30 and 1.60 g/L.

<table>
<thead>
<tr>
<th>Correctly classified, %</th>
<th>L-LDL</th>
<th>D-LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.30 g/L</td>
<td>68*</td>
<td>98</td>
</tr>
<tr>
<td>1.30–1.59 g/L</td>
<td>57*</td>
<td>93</td>
</tr>
<tr>
<td>≥1.60 g/L</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Significantly different from D-LDL by McNemar test, P < 0.001.

Table 4. Comparison of LDL-C concentrations determined by L-LDL, D-LDL, and BQ-LDL in type III patients (median and range).

<table>
<thead>
<tr>
<th></th>
<th>L-LDL</th>
<th>D-LDL</th>
<th>BQ-LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median, g/L</td>
<td>2.72*</td>
<td>0.84*</td>
<td>1.03</td>
</tr>
<tr>
<td>Range, g/L</td>
<td>1.20–6.12</td>
<td>0.42–2.94</td>
<td>0.68–3.18</td>
</tr>
</tbody>
</table>

* Significantly different from BQ-LDL by Wilcoxon signed rank test, P < 0.05.

Table 5. Effect of pre- and postprandial state on Tg, cholesterol, and LDL-C concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Nonfasting</th>
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<tbody>
<tr>
<td>Tg</td>
<td>1.18 ± 0.56*</td>
<td>1.87 ± 1.10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.71 ± 0.33</td>
<td>1.70 ± 0.32</td>
</tr>
<tr>
<td>L-LDL-C</td>
<td>1.14 ± 0.30</td>
<td>1.13 ± 0.32</td>
</tr>
<tr>
<td>D-LDL-C</td>
<td>1.01 ± 0.28</td>
<td>0.96 ± 0.28</td>
</tr>
<tr>
<td>BQ-LDL-C</td>
<td>1.12 ± 0.29</td>
<td>1.08 ± 0.28</td>
</tr>
</tbody>
</table>

* Significantly different from nonfasting Tg concentration by paired t-test, P < 0.001.
precision was excellent (<3%), but the interassay precision, especially at lower concentrations of LDL-C, was less than desirable (CV <8%). When we compared LDL-C values determined by L-LDL against the reference method, we found significant differences across all TG ranges (both normo- and hypertriglyceridemic, \( P < 0.001 \)).

With L-LDL, 103 of the total 156 patients (including 47 of 50 with \( \text{TG} \geq 4 \text{ g/L} \)) had LDL-C values that showed a >10% bias as compared with BQ-LDL. In contrast, D-LDL values did not have any significant difference with the BQ-LDL values across the overall TG range and in normotriglyceridemic samples. Although the D-LDL assay showed significantly increased values in hypertriglyceridemic sera, this was not as great as with the L-LDL assay. Moreover, other authors have also shown a definite positive bias for D-LDL estimation compared with BQ-LDL in hypertriglyceridemia \([13, 14]\), but in all cases the bias was less than the NCEP total error goal of <12%. In this study we found the mean absolute bias for D-LDL in samples with \( \text{TG} \geq 4 \text{ g/L} \) to be 12.5%. For the same patients, L-LDL gave a mean absolute bias of 30.6% (with two of the values actually having >100% bias compared with BQ-LDL). For samples with \( \text{TG} < 4 \text{ g/L} \), D-LDL had a mean absolute bias of 6.2% as compared with 12.7% for L-LDL. However, because L-LDL and D-LDL were performed on serum samples and BQ-LDL was performed on EDTA plasma samples, the actual biases for the two methods are 3% lower than that reported. Thus, our comparison studies clearly demonstrate that L-LDL gives less precise and less accurate results in normotriglyceridemic patients as compared with D-LDL, and this difference becomes accentuated in cases with hypertriglyceridemia.

The NCEP LDL-C cutoff concentrations of 1.30 and 1.60 g/L are very important laboratory parameters in therapeutic decision making. By reporting a patient’s LDL-C above or below the conventional cutoffs, the laboratorian is conveying a definite message to the clinician about risk stratification. Hence, having an assay that can reliably and consistently classify patients in the NCEP categories of LDL-C concentrations <1.30, 1.30–1.59, and ≥1.60 g/L is absolutely mandatory. Comparison studies for the two methods show D-LDL to be obviously superior to L-LDL as it appropriately classified >90% of patients with LDL-C concentrations <1.30 and 1.30–1.59 g/L. L-LDL performed rather poorly in these two categories. The only occasion when L-LDL did compare with D-LDL was in patients with LDL-C ≥1.60 g/L (100% appropriate classification for both), which we believe is because of the tendency of L-LDL to overestimate LDL-C in most cases. If these percentages were translated into numbers it would mean that 48 of the total 156 patients were misclassified by L-LDL (with two patients actually skipping categories) as compared with 7 of 156 patients being misclassified by D-LDL (with no patient skipping categories). It is obvious from the above figures that any laboratory using L-LDL for risk assessment as well as follow-up would have to contend with these major discrepancies.

Having established the superiority of D-LDL over L-LDL as an assay with regard to analytical precision, accuracy, and appropriate classification, we examined their usefulness in the subset of patients with type III hyperlipidemia. Our results show that L-LDL is an extremely inaccurate test in these patients, with the median LDL-C being 2.74 ± 1.62 as compared with 1.23 ± 0.88 g/L for BQ-LDL. One of the possible reasons to explain this discrepancy could be that the LDL precipitating reagent used in L-LDL is also precipitating other cholesterol-rich fractions into the filtrate. In contrast, D-LDL, being an immunoprecipitation assay, performed better as reported previously \([10]\).

Calculation of LDL-C with the Friedewald equation requires patients to fast for 10–12 h \([12]\). Any assay that could dispense with fasting samples would not only be convenient for the patient at large but would also prove beneficial in subsets such as diabetics. The L-LDL and D-LDL both showed good correlation between fasting and postprandial samples. Other authors who have worked with D-LDL have shown a similar correlation and recommended its use irrespective of the patient’s fasting status \([10, 13, 14]\). We felt it was paradoxical that L-LDL, which showed poor results at higher TG concentrations, should show such good correlation in the fast-feed study. A plausible reason for this observation is that only one of the 25 subjects attained a TG value > 4 g/L after the test meal.

One criterion by which some laboratories could adopt the L-LDL in the calculation of LDL-C is that it claims to be a cheaper assay. The immunoprecipitation method used by D-LDL costs $750 for a 100-test kit. In contrast, the L-LDL assay has been priced at $620 for a 125-sample kit. However, the L-LDL controls, which have to be bought separately, cost $75 to the laboratory and are stable for only 10 days. Obviously, a more detailed scrutiny of the “fiscal benefits” in using the L-LDL assay needs to be done.

In conclusion, the purpose of this study was to compare two methods for measuring LDL-C in the serum against the reference method and establish the incremental benefit (if at all) of one over the other. We feel that D-LDL has definitely emerged as a superior assay over L-LDL in terms of precision, accuracy, and correct classification of patients. However, we would caution at this point that any laboratory that adopts a new cholesterol assay must participate in a proficiency program such as ALERT to keep a constant vigilance on the quality of the results being reported. We also feel the traditional cutoff of TG < 4 g/L for using the Friedewald equation may need to be revisited. In this context, McNamara et al. \([12]\) have clearly shown that even in samples with TG ≥2 g/L the Friedewald equation results in significant inaccuracies (>10% bias in 23% and 41% of samples with TG 2.01–3
g/L and 3.01–4 g/L, respectively). In addition, a recent study from this center has demonstrated the greater accuracy of D-LDL over Friedewald equation in the diabetic population with Tg concentrations <4 g/L [18]. Normotriglyceridemic nonfasting patients are the only subset of patients where either the D-LDL or L-LDL assay can be used. However, on the basis of the findings of this study, the authors would favor the D-LDL assay over L-LDL. In hypertriglyceridemic patients (fasting or nonfasting), the D-LDL assay could be used in most clinical laboratories. The L-LDL is too inaccurate to be a reliable assay in these patients. We did not evaluate patients with Tg >10 g/L in this study for two reasons. First, the L-LDL manufacturer does not recommend the assay in samples with Tg >10 g/L. Second, and more importantly, we strongly believe that in patients with such high Tg concentrations, instead of spending time and effort in determining LDL-C, the prudent clinician would be more concerned in lowering the Tg value and with it the attendant immediate risk of pancreatitis before assessing cardiovascular risk. Thus, on the basis of our data, we believe that the D-LDL immunoprecipitation assay is the preferred method for measurement of LDL-C over the L-LDL method.

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References