Hypercholesterolemia is one of the major risk factors associated with coronary heart disease (CHD). Although genetics partly dictates a person's cholesterol level, diet and medication also may have an effect. To assess an individual's risk of CHD and to treat patients with hypercholesterolemia, it is crucial that the values for the major serum lipids are accurate. These values include total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides.

Total cholesterol is composed of HDL, LDL, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), and lipoprotein (a) [Lp(a)]. About 60% to 70% of total cholesterol is found in the LDL fraction. Low-density lipoprotein deposits cholesterol in tissue, creating plaque that clogs coronary arteries; high LDL values are consistent with increased CHD risk. For this reason, LDL is termed the “bad cholesterol.” High-density lipoprotein levels are inversely proportional to CHD risk; high HDL serum levels are thought to protect against CHD because HDL removes cholesterol from tissue and delivers it to the liver where it is metabolized into bile acids and ultimately excreted. High-density lipoprotein therefore is called the “good cholesterol.”

Coronary heart disease is a complex, multifactorial condition. Some risk factors, such as gender, age, and genetic predisposition for developing CHD, cannot be modified. Other risk factors, such as high cholesterol levels, hypertension, and cigarette smoking, can be modified to decrease the risk of CHD. The National Cholesterol Education Program (NCEP) encourages everyone to “Know your number”—your TC number, that is—and to be aware of your CHD risk, as illustrated in Table 1. Initial classification of CHD risk is based on screening for TC, but LDL becomes the key for dietary and drug treatment.

**ABSTRACT**

Low-density lipoprotein (LDL) cholesterol is a critical lipid subfraction because it is linked directly with the risk of coronary heart disease (CHD). Current approaches to diagnosing and monitoring CHD emphasize the significance of accurate, precise LDL values. Due to the inherent complexity of measuring LDL by the reference method, ultracentrifugation, it usually is estimated by a calculation that uses measured values for total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Calculated LDL (CLDL) results may be inaccurate, especially if a patient’s triglyceride level is elevated, and it requires a specimen from a patient who has fasted. New assays allow for the direct measurement of LDL (DLDL) analogous to assays for HDL and do not require the patient to fast. While CLDL typically compares well with ultracentrifugation and DLDL, it may provide a result that is different in some instances; that difference may have clinical significance. A method comparison study of CLDL and DLDL resulted in the following linear regression equation:

\[
DLDL = 1.067 \times CLDL + 1.597, \quad r = 0.974, \quad n = 36.
\]

The NCEP’s cut-point numbers are, of necessity, oversimplified. Acceptable, safe lipid values vary among individuals and should be interpreted in light of the total risk factor picture for every patient. The key lipid values, however, are a convenient way to educate the general public about the relationship between lipids and CHD.

The NCEP takes a conservative approach and does not recommend instituting treatment to lower cholesterol levels on the basis of a single elevated value. Lipid analyses should be repeated to allow for the many variables that can cause elevations. As the biologic variability of LDL is about 9.5%, some investigators have recommended as
TABLE 1. NATIONAL CHOLESTEROL EDUCATION PROGRAM KEY LIPID VALUES FOR THE INITIAL CLASSIFICATION OF THE RISK OF CORONARY HEART DISEASE*

<table>
<thead>
<tr>
<th>Interpretation/Classification</th>
<th>TC</th>
<th>LDL</th>
<th>HDL</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormally low level</td>
<td>&lt; 34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desirable level</td>
<td>&gt; 35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline high level</td>
<td></td>
<td>200-400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High level</td>
<td></td>
<td>400-1,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very high level</td>
<td></td>
<td>&gt;1,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk CHD</td>
<td>&lt; 200</td>
<td>&lt;130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline high-risk CHD</td>
<td>200-239</td>
<td>130-159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk CHD</td>
<td>&gt; 240</td>
<td>&gt;160</td>
<td>400-1,000</td>
<td></td>
</tr>
</tbody>
</table>

*All values are expressed as mg/dL. TC indicates total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; CHD, coronary heart disease.

many as four LDL determinations before making treatment decisions. Repeatedly high LDL values indicate the need to initiate dietary or drug therapy to lower blood cholesterol.

Low-density lipoprotein is measured by three methods:
1. Ultracentrifugation
2. Calculation
3. Direct assay

Although ultracentrifugation (β quantification) is the reference method, it is impractical for routine clinical use owing to its complexity, the time required (18 hours at 105,000 × g), and the need for special equipment. Clinical laboratories typically use the Friedewald formula to calculate an LDL value (CLDL, for short). Calculated LDL is derived from the measured values of TC, HDL, and triglycerides as follows:

\[ \text{LDL} = \text{TC} - \left(\frac{\text{HDL} + \text{triglycerides}}{5}\right) \]

The amount of VLDL roughly equals the triglycerides divided by 5. DeLong and colleagues developed an alternate calculation to correct for high triglyceride levels; the calculation divides the triglyceride concentration by 6. Usually, a CLDL value is reliable and clinically useful, and the calculation of LDL is an acceptable and established practice. A CLDL value may lack precision and accuracy, however; an excessive imprecision and bias is associated with each of the measured lipid values. The relationship between VLDL and triglycerides is nonlinear and starts to show problems in the 200 to 400 mg/dL range, falling off at the higher triglyceride levels. A high degree of variability associated with CLDL makes it difficult to detect small changes in LDL. Specimens also must be from patients who have fasted because recent meal ingestion affects the serum triglyceride level. Inaccurate LDL results can lead to the miscalculation of CHD risk. An individual with a falsely elevated LDL value may be given unnecessary lipid-lowering therapy, or an individual with a falsely low LDL may not be started on a therapeutic regimen to lower the risk of CHD.

Recently, direct LDL (DLDL, for short) methods have been introduced. An apolipoprotein antibody separates the LDL from other lipid fractions. Once isolated, LDL cholesterol is measured using a routine TC method. Direct LDL does not require a specimen from a fasting patient, and it correlates well with the ultracentrifugation reference procedure. We compared the traditional CLDL with a DLDL method for specimens submitted to our laboratory for lipid profile analysis.

Materials and Methods
Total Cholesterol Assay
The Boehringer Mannheim (Indianapolis) cholesterol/hp reagent was used following the manufacturer’s directions. The kit is based on a sequential cholesterol esterase/cholesterol oxidase/peroxidase modified Trinder method. The assay was performed using a Hitachi 717 analyzer (Boehringer Mannheim). Typical day-to-day coefficients of variation (CVs) for this assay range from 2.5% to 3.5%.

High-Density Lipoprotein Assay
The Boehringer Mannheim HDL cholesterol kit was used following the manufacturer’s directions. The procedure uses phosphotungstic acid to precipitate LDL and VLDL in a button after centrifugation. High-density lipoprotein in the supernatant is measured using the TC assay described above. Typical day-to-day CVs for this assay range from 3% to 4%.

Triglycerides Assay
The Boehringer Mannheim triglycerides/GPO kit was used following the manufacturer’s directions. This procedure uses a sequential lipase/glycerol kinase/glycerol phosphate oxidase/peroxidase method. The assay was performed using a Hitachi 717 analyzer. Typical day-to-day CVs for this assay range from 2.5% to 4.5%.
Calculated Low-Density Lipoprotein
Calculated LDL was performed using the TC, HDL, and triglycerides values using the Friedewald formula as described earlier.

Direct Low-Density Lipoprotein Assay
The LDL cholesterol test, developed by Genzyme (Cambridge, Mass) and distributed by Sigma (St Louis), was used. The test features an affinity-purified goat polyclonal antibody, immobilized on polystyrene beads, that binds HDL and VLDL, but not LDL. The assay separates LDL from the chylomicrons, VLDL, and HDL using an apolipoprotein antibody-based filtration system. The assay combines 200 μL of reagent and 30 μL of serum in a plastic inner tube. The inner tube then is placed in the outer tube, and both are centrifuged for 5 minutes. The inner tube, containing the beads, is discarded and the LDL filtrate in the bottom of the outer tube is tested for cholesterol using the routine cholesterol procedure. Day-to-day CVs for low and high controls, mean values 92 and 193 mg/dL, are 1.08% and 3.8%, respectively.

Method Comparison
Patient specimens were assayed for TC, HDL, triglycerides, CLDL, and DLDL. The CLDL and DLDL values were compared using linear regression analysis, with CLDL designated as the reference method and DLDL as the test method.

Specimens
Specimens submitted from the facilities routinely served by our laboratory were tested. Of a total of 45 specimens from 13 men (ages 28–81) and 32 women (ages 26–86), 9 patients’ data were excluded from linear regression analysis due to high triglyceride values or disparity between CLDL and DLDL results. Blood was collected in serum separator vacutainer tubes. Specimens were shipped refrigerated and stored from 1°C to 8°C in the laboratory until testing.

Results and Discussion
The linear regression plot of CLDL vs DLDL is shown in the Figure. Calculated LDL and DLDL compare well for this set of patients, with CLDL values about 1.6 mg/dL lower than DLDL values. The data points included patients with triglyceride values up to 335 mg/dL; values from patients above 400 mg/dL triglycerides were excluded. Table 2 shows that for some individuals (A–C), CLDL and DLDL values are nearly identical with less than 5% difference between them. In other cases (D–F), the agreement is reasonably good, but differences between 5% and 13% are noted, and some potential for clinical misinterpretation exists. Patient D had a CLDL of 116 but a DLDL of 131. Using the NCEP LDL cut-point of 130, there is a low risk of CHD by CLDL but borderline high risk by DLDL.

The discrepancies between CLDL and DLDL are illustrated best by the nine patients whose data were withheld from the method comparison study (Table 3). Five of the patients have hypercholesterolemia with a potential high risk for CHD, and four have values below 200 mg/dL. Six have borderline high triglyceride values, three have high values, and one has a very high value. Differences between CLDL and DLDL vary from a low of 20% to a high of 260%. The most dramatic case is patient 2, for whom it is impossible to obtain even a CLDL in this case because the estimated value is −12 mg/dL (due to the very high triglyceride and very low HDL values), whereas the DLDL is 56 mg/dL. The disparity between CLDL and DLDL values would result in different assessments of the risk for CHD or for clinical decisions when monitoring patients on dietary and/or drug therapy for the purpose of lowering cholesterol levels. Note that 9 (20%) of the patients in this small study population showed considerable differences between CLDL and DLDL. The high percentage of discrepancies likely reflects the makeup of the study group—primarily elderly patients with a documented or suspected pathologic condition that resulted in a request for a complete lipid profile. Fewer differences would be expected in a younger, healthy population.
Other workers also have evaluated the Sigma DLDL assay.\(^8\)\(^-\)\(^1\(^1\) McNamara and colleagues compared the DLDL assay with ultracentrifugation.\(^8\) Correlation between the assays was very good \((r = .97, n = 100)\), with a slight negative bias for normal subjects and a small positive bias for hypertriglyceridemic patients. Between-run CVs ranged from 2.0% to 5.1%. Proficient laboratories that maintain a high degree of precision for their TC, HDL, and triglyceride assays may generate CLDL with a variability of less than 5%. Coupled with a biologic variability of about 6% to 10%, total variability of CLDL values is probably in the range of at least 11%.\(^1\(^2\) To monitor the efficiency of cholesterol-reducing therapeutic regimens via LDL, a method should have a CV of less than 5%. Calculated LDL values may vary from 11% to 26%, whereas the direct LDL can attain a CV of less than 4% and is much preferable for detecting decreases in LDL in response to therapy.\(^1\(^2\)

Ransdell and colleagues found day-to-day CVs to range from 3.2% to 6.2% and good correlation with the CLDL for specimens less than 400 mg/dL triglycerides \((n = 42, \text{slope} = 1.067, r = 0.912)\), but the correlation for specimens greater than 400 mg/dL triglycerides was unimpressive \((n = 33, \text{slope} = 0.202, r = 0.332)\).\(^9\) Singh and colleagues found DLDL day-to-day precision to be 3% and correlation with specimens from 42 to 400 mg/dL triglycerides to be acceptable \((n = 142, \text{slope} = 0.9, r = .88)\), with a linear range of 25 to 270 mg/dL.\(^1\(^0\) Castellani and Ghadiali also found that the DLDL correlates well with the CLDL for specimens with less than 400 mg/dL triglycerides \((\text{slope} = 0.986, r = .966)\).\(^1\(^1\)

The Genzyme assay marketed by Sigma and evaluated here is also available from Pointe Scientific (Lincoln Park, Mich). Performance of the Pointe assay should be similar to the Sigma product. We anticipate that other manufacturers will offer immunoassay-based procedures for LDL in the future. Helena Laboratories (Beaumont, Tex) offers an electrophoretic assay for DLDL, based on the electrophoretic, rather than immunosorbert, separation of the lipid subfractions. Although the automated procedure simultaneously measures HDL and LDL, it is necessary to perform a chemical measurement of total cholesterol to calculate the amount of HDL and LDL using the electrophoretic data.

Calculated LDL is used routinely and provides a useful estimate of LDL for most patients. About 90% of CLDL values from typical patients are within about 10% of ultracentrifugation values.\(^1\(^3\) Calculated LDL still can be used reliably for most individuals whose triglyceride concentration is less than 400 mg/dL. The DLDL assays may be useful in some cases because they offer greater precision and accuracy than CLDL, are not affected by elevated triglycerides, and do not require a fasting specimen. Cole and Ferguson reported that the Sigma DLDL test is unaffected by triglyceride concentrations up to 1,100 mg/dL.\(^1\(^4\) After a patient is identified as hypercholesterolemic and lipid-lowering therapy is instituted, the success of the therapeutic regimen may be monitored by LDL values alone. The NCEP recommends measuring LDL every 4 months during the first 18 months of therapy and then biannually. Drawbacks to DLDL testing include the relatively high reagent cost (CLDL costs nothing because it is a calculated value), additional labor and time to perform the assay, and potential problems with reimbursement from Medicare (CLDL has not been reimbursed by Medicare since 1992).

Calculated LDL values depend on the accuracy and precision of the three individual lipid assays required for the calculation, require a fasting specimen, and are not recommended in the presence of chylomicrons, when triglycerides exceed 400 mg/dL, or for patients with Type III hyperlipoproteinemia.\(^1\) McNamara and colleagues found that even when triglycerides are less than 200 mg/dL, about 14% to 16% of the population may be potentially misclassified on the basis of LDL when using the Friedewald formula.\(^7\) Between 300 and 400 mg/dL triglycerides, 41% of specimens tested deviated by greater than 10% from ultracentrifugation values and above 400 mg/dL, more than 57% of CLDL values disagreed by greater than 10%. The CLDL is most prone to misclassification for individuals close to the NCEP cut-points and persons who have not fasted 12 to 14 hours. A rule of thumb suggests that a

### TABLE 2. LIPID VALUES FOR SELECTED PATIENT SPECIMENS USED IN THE METHOD COMPARISON STUDY*

<table>
<thead>
<tr>
<th>Patient</th>
<th>TC</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>CLDL</th>
<th>DLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>137</td>
<td>70</td>
<td>43</td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td>B</td>
<td>163</td>
<td>109</td>
<td>25</td>
<td>116</td>
<td>118</td>
</tr>
<tr>
<td>C</td>
<td>214</td>
<td>158</td>
<td>39</td>
<td>143</td>
<td>150</td>
</tr>
<tr>
<td>D</td>
<td>171</td>
<td>59</td>
<td>43</td>
<td>116</td>
<td>131</td>
</tr>
<tr>
<td>E</td>
<td>223</td>
<td>241</td>
<td>52</td>
<td>123</td>
<td>130</td>
</tr>
<tr>
<td>F</td>
<td>167</td>
<td>282</td>
<td>16</td>
<td>95</td>
<td>109</td>
</tr>
</tbody>
</table>

*All values are expressed as mg/dL. TC indicates total cholesterol; HDL, high-density lipoprotein; CLDL, calculated low-density lipoprotein; DLDL, direct low-density lipoprotein.
1% decrease in LDL decreases the incidence of CHD by 2%. Another measure of CHD risk is the LDL to HDL ratio, another reason for using the more accurate DLDL instead of CLDL.

The problem of semantics raised by the new DLDL assays should be recognized. Unless specifically clarified, LDL may refer to either a calculated LDL or a direct LDL. Here, we have used CLDL and DLDL as unambiguous abbreviations for the two types of LDL. Laboratories should specify how LDL values are derived to avoid confusion. Some laboratories may report both CLDL and DLDL in accordance with clients’ requests or internal testing policies. For example, if the TC, HDL, and triglyceride values are normal, a CLDL may automatically be performed as a reflex test. The LDL situation is now similar to that for free thyroxine (FT₄). A variety of direct FT₄ assays are now available, and many endocrinologists recommend a FT₄ and sensitive thyroid-stimulating hormone (TSH) for thyroid screening. FT₄ also may refer to a calculated estimate of the FT₄ concentration, that is, the free thyroxine index (FTI) derived from the T₄ uptake and total T₄ values. Although both the CLDL and FTI are estimations calculated from measured values, they may be totally acceptable for diagnosis and monitoring of patients. The debate about when to measure LDL directly as opposed to calculating it most likely will continue.

**Conclusion**

Direct LDL assays represent an important advance in the ability to provide accurate and precise values for a significant lipid subfraction. Direct LDL assays will likely be routinely used when analyzing specimens from individuals who have been diagnosed with coronary heart disease or for whom a CLDL value cannot be calculated due to elevated triglycerides. Low-density lipoprotein values will probably continue to be calculated for most patients as they are typically valid estimates, providing that the assumptions implicit in the Friedewald equation hold, and they require no additional effort or expense.

**References**


