Variability of Lipid Measurements: Relevance for the Clinician

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Decreasing the large test variability associated with measurements of blood cholesterol, triglyceride, and high-density lipoprotein (HDL)- and low-density lipoprotein (LDL)-cholesterol is likely to improve the classification of coronary heart disease (CHD) risk and allow improved monitoring of lipid-lowering treatments. However, improving test precision will benefit the clinician only if (a) the analytical test variability is high relative to the biological test variability and (b) detecting subtle responses to diet or drug therapy is clinically important. Improving HDL- and LDL-cholesterol test precision can be expected to increase the clinical usefulness of these measurements because values for HDL- and LDL-cholesterol correlate closely with CHD risk; are associated with small, yet clinically important, changes in response to diet and (or) drug therapy; and have substantial analytical test variability relative to biological variability. On the other hand, measurements of both blood cholesterol and triglyceride have high biological relative to analytical variability, and do not correlate as closely with CHD risk. Therefore, further improvements in precision for these measurements are less likely to be useful to the clinician.

Indexing Terms: cholesterol • triglycerides • accuracy • precision • heart disease • lipoproteins

Each year, >500,000 people in the US die from coronary heart disease (CHD).\(^4\) Cigarette smoking, hypertension, and hypercholesterolemia are the three principal preventive risk factors for this disease. In 1984, the National Heart, Lung and Blood Institute convened a consensus panel that recommended lower cholesterol threshold values to trigger treatment of hypercholesterolemia. The National Cholesterol Education Program (NCEP), created by the Institute in 1985 to “reduce the prevalence of elevated blood cholesterol in the United States” (1), carried the recommendations of the 1984 consensus panel one step further.

The report of the Adult Treatment Panel of the NCEP \(^2\) represented a major change over earlier guidelines for several reasons. First, low-density lipoprotein (LDL) cholesterol was recognized as a more sensitive predictor of CHD risk than total cholesterol, and assay of total cholesterol was largely relegated to the status of a screening test. Use of LDL-cholesterol assay was recommended to evaluate CHD risk and to monitor response to diet and (or) drug therapy. Second, treatment goals were well defined, encouraging the use of frequent laboratory tests to determine whether such goals had been achieved. Finally, the clinical repercussions of test inaccuracy and imprecision were recognized, and a laboratory standardization panel was created with the aim of improving accuracy and precision of cholesterol measurements \(^3\). These guidelines, widely publicized, stimulated mass cholesterol screening programs around the country. According to NCEP guidelines, >41% of American adults were estimated to have abnormally high screening values for cholesterol and to require further evaluation \(^4\).

These recommendations placed increased demands on total cholesterol, serum triglyceride, and high-density lipoprotein (HDL) cholesterol measurements to be as accurate and reproducible as possible. Imprecise measurements will impair the correct classification of CHD risk, increasing the frequency of either over- or under-treatment. Further, high test variability may limit the clinician’s ability to detect an important response to therapy or to determine a meaningful reduction in CHD risk.

Our purpose here is to assess whether the analytical and biological variability usually associated with these lipid measurements interferes with their clinical usefulness, and to evaluate the extent that reductions in test variability will improve the clinician’s ability to evaluate and treat lipid disorders.

Lipid and Lipoprotein Test Variability: Definition and Clinical Significance

The test variability associated with lipid and lipoprotein measurements has two components. The first component is the natural biological variability \((V_b)\), which may be influenced by many factors, including recent diet, day of the week or season, illness, and postural changes (Table 1). The second component is the analytical variability \((V_a)\) due to the imprecision of the assay. These values are usually expressed as a coefficient of variation \((CV_a, CV_b)\) around the test mean. The total test variability is also expressed as a coefficient of variation \((CV_t)\). The total test variability \((CV_t)\) is defined \((5)\) as \((CV_t)^2 = (CV_a)^2 + (CV_b)^2\).

To reduce the \(CV_t\) of any test, three approaches can be considered. First, the \(CV_t\) can be decreased by improving the reliability of the assay in the clinical laboratory. The Laboratory Standardization Panel of the NCEP has set standards for total-cholesterol measurements \(^3\), and others have suggested that similar requirements be established for other lipid measurements \(^7\). Figure 1

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\(^3\) Nonstandard abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; CHD, coronary heart disease; NCEP, National Cholesterol Education Program; CV\(_a\), total test variability; CV\(_b\), analytical variability; CV\(_t\), biological variability; and apo, apolipoprotein.

Received December 4, 1992; accepted February 3, 1993.
shows the effects of reducing $CV_a$ on the $CV_r$ for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, in which it is assumed that the $CV_b$ for each test remains constant. As illustrated in Figure 1, decreasing $CV_a$ improves $CV_r$. However, a point is reached for each lipid measurement where further improvements in test precision no longer significantly reduce $CV_r$. For total-cholesterol and triglyceride measurements, where the $CV_a$ achieved in many clinical laboratories is reported at 3% or less, further improvements in test precision do not yield meaningful benefits in $CV_r$. On the other hand, the $CV_a$ reported for HDL- and LDL-cholesterol measurements currently is high relative to $CV_b$, and further reductions in test imprecision decrease $CV_r$ and improve test performance. To improve test precision, an alternative to reducing $CV_a$ directly is to perform the assay in duplicate or triplicate and report the mean value—an approach commonly used in the research setting, but rarely cost-effective when routinely used in the clinical laboratory.

A second approach to reduce total test variability is to decrease $CV_b$, such as by assessing differences in patients’ characteristics or behavior that may affect repeated lipid measurements (Table 1). Third, serial specimens can be drawn 1 or 2 weeks apart and the individual values averaged. The extent to which this approach will decrease test variability can be estimated by dividing $CV_r$ by the square root of the number of repeated samples (8). Acknowledging the large variability of total- and LDL-cholesterol measurements, the NCEP encouraged that mean values derived from at least two measurements be obtained before classifying a patient at high, borderline, or low risk for CHD (2).

Because the total test variability of most lipid measurements is large, the physician should be aware of the $CV_r$ and the potential impact that this variance may have on clinical decision-making. Reliable measurements may be particularly important when:

(a) **there is a strong correlation with CHD risk.** Because a primary goal of lipid-disorder management is to reduce CHD risk, an accurate assessment of risk reduction in response to treatment is necessary. Therefore, the clinician may accept a larger $CV_r$ for blood triglyceride values than for HDL-cholesterol values, which correlate much more strongly with CHD risk. On the other hand, even small changes in HDL-cholesterol may convey important information regarding CHD risk reduction and therefore may be clinically important.

(b) **the effect of therapy is expected to be relatively small.** For example, to assess a patient’s response to diet, the clinician may need to detect a decrease in LDL-cholesterol of only 10%. For one to reliably discern this difference from baseline measurements (assumed, for the sake of simplicity, to reflect the patient’s true LDL-cholesterol value), the $CV_r$ would need to be less than one-half of the expected response to therapy, or <5%. A 10% reduction in LDL-cholesterol in response to diet would then reflect a true difference from baseline within the 95% confidence interval of the test. This simple approach to estimating the $CV_r$ necessary to assess response to treatment will be helpful for individuals who respond at least as well as expected to the intervention. A lower $CV_r$ may still be required to detect an effect of the intervention for those who show less than average responses.

(c) **the test is used to monitor response to therapy, rather than to classify CHD risk status.** In the latter setting, repeat measurements are more practical because they are usually required only during the initial evaluation of the patient. However, when a test is used to monitor response to therapy, multiple measurements may be required after each therapeutic change. In our own clinic, less than half of patients achieve goal LDL-cholesterol concentrations after treatment with their initial therapeutic regimen, and several drugs and (or) dose changes are necessary until an optimal response is achieved.

Obtaining repeat measurements after each therapeutic intervention is inconvenient, time-consuming, and expensive and may be difficult for the physician in

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**Table 1. Factors Affecting the Biological Variability of Lipid Measurements**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Clinical Information</th>
</tr>
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<tbody>
<tr>
<td>Smoking</td>
<td>Day of week</td>
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<tr>
<td>Exercise</td>
<td>Seasonal variations</td>
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<tr>
<td>Medication changes</td>
<td>Fasting status</td>
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<tr>
<td>Illness</td>
<td>Alteration of diet habits</td>
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<tr>
<td>Pregnancy</td>
<td>Weight change</td>
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<tr>
<td>Postural changes</td>
<td>Alcohol intake</td>
</tr>
<tr>
<td>Tourniquet time</td>
<td>Conditions of specimen storage</td>
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*For an excellent detailed review, see Cooper et al. (5).*

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**Fig. 1. Effect of test precision on total variability for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol**

Assumes biological variability for blood triglycerides (top line) = 21%, LDL-cholesterol (second line from the top) = 8.8%, total cholesterol (third line from the top) = 6.5%, and HDL-cholesterol (bottom line) = 4.8%. The expected analytical variability frequently achieved in clinical laboratories is shown by the x for each laboratory test.
private practice to do. Therefore, reducing test variability to obviate the need for multiple measurements is a desirable goal.

Interpreting Test Variability: Implications for Measurement of Total Cholesterol, Triglyceride, and HDL- and LDL-Cholesterol

Total Cholesterol

Importance. Although values for total cholesterol now are rarely used to monitor response to therapy, careful attention to accuracy and precision of the total cholesterol measurement remains important for several reasons. First, it is the primary screening tool for detecting hypercholesterolemia, therefore suboptimal accuracy and precision will result in significant misclassification and result in normcholesterolemic individuals receiving diet and (or) drug therapy, and in hypercholesterolemic individuals not receiving correct treatment (9, 10). Second, most clinical laboratories use the Friedewald equation to calculate LDL-cholesterol (11). Because this formula includes dividing the triglyceride value by five, and because values for HDL-cholesterol are usually less than a third of total-cholesterol values, the variability of the total-cholesterol measurement usually contributes a large part of the variability observed for LDL-cholesterol. Therefore, accurate decisions regarding classification of CHD risk and treatment of above-normal LDL-cholesterol concentrations depend upon reliable total-cholesterol measurements. For these reasons, the laboratory standardization panel was established to set appropriate accuracy and precision standards for total cholesterol (3). Acceptable standards for inaccuracy and imprecision were considered to be <5%, with further reductions to 3% by 1993.

Variability. Figure 1 shows the effect of increasing analytical precision of total-cholesterol measurements on the total variability. Assuming a biological CV of 6.5% (12), decreasing the CVa from 5% to 3% will reduce the CVb by 1.2%. However, when the analytical variability is lowered to <3%, negligible reductions in total test variability are achieved.

Figure 2 shows the effect of replicate measurements on the CVa, assuming a test precision of 3%. At this level of precision, the analytical variability contributes only 18% of the total test variability, and therefore replicate measurements do not significantly reduce total test variability. On the other hand, drawing additional specimens to reduce the CVa has much greater impact.

Factors affecting the biological variability of lipid and lipoprotein measurements are shown in Table 1. Careful attention to patients' characteristics and blood-drawing and specimen-handling procedures can be helpful to reduce preanalytical and (or) biological variability. Large interpersonal fluctuations in these values can sometimes be explained by between-sampling changes in the patients' behavior or lifestyle, or by differences in blood-drawing procedures, information that can be elicited either from the patient or laboratory personnel (preanalytical variability). However, the extent to which these factors affect lipid and lipoprotein measurements is usually difficult to quantify in the individual patient.

In summary, many laboratories have been able to decrease analytical variability to <3% for total-cholesterol measurements. Further limiting analytical variability may still significantly improve test performance (Table 1). Attention to uniform blood-drawing procedures and specimen handling may also be helpful. Alternatively, multiple samplings of serum cholesterol may partly overcome this biological variability. Reducing total test variability to <6% by improving test precision (Figure 2) should lead to fewer misclassification errors when individuals are evaluated according to NCEP guidelines (10). However, the clinical significance of further improvements in test performance is unclear.

Triglycerides

Importance. Evaluation of serum triglycerides is important for three reasons. First, values exceeding 11.3 mmol/L (1000 mg/dL) are associated with pancreatitis, and individuals with values >5.65 mmol/L (500 mg/dL) should be identified and targeted for evaluation and therapy (2). Second, in patients with milder increases of blood triglycerides (2.80–5.65 mmol/L; 250–500 mg/dL), who have familial combined hyperlipidemia or concomitant abnormalities in LDL and (or) HDL concentrations, diet and (or) drug therapy can be considered to reduce CHD risk (2). In such patients, serial triglyceride determinations are necessary to monitor response to therapy. Third, values for serum triglyceride provide an estimate of very-low-density lipoprotein (VLDL)-cholesterol.
terol concentrations, which are necessary for the calculation of LDL-cholesterol.

VLDL from frankly hypertriglyceridemic subjects has been shown to convert monocytes to foam cells and is toxic to endothelial cells (13); therefore, abnormal VLDL has atherogenic properties, at least in vitro. Blood triglyceride values are also associated with CHD in most prospective epidemiological studies. However, the extent to which this relationship exists independently of HDL-cholesterol is unclear (14). In most studies, the association between high triglyceride values and CHD loses significance when HDL-cholesterol and either total- or LDL-cholesterol are included in a multivariate analysis. Intervention trials do not show a relationship between triglyceride reductions and decreased CHD outcomes (15–17). For these reasons, LDL- and HDL-cholesterol, rather than blood triglyceride concentrations, are used to classify and monitor CHD risk status.

Variability. Blood triglyceride measurements are associated with much biological variability, and have a CVb usually >20% (7, 18–20). Because the CVb of the test in most clinical laboratories is usually <5% (7), >95% of the total test variability is ascribable to the biological variability. Therefore, further improving the test precision has little effect on reducing CVb (Figure 1). Drawing multiple samples, rather than performing replicate measurements on the same specimen, is an effective approach to reduce CVb (Figure 3).

Drug therapy effectively diminishes blood triglyceride concentrations. Gemfibrozil may reduce triglyceride concentrations by >40% (15). In hypertriglyceridemic subjects, niacin and fish oil decrease values for blood triglyceride by >25% (21, 22). To reliably detect response to an agent that decreases values by 25%, a test with a CVb of <12.5% would be desirable, according to the criteria discussed earlier. To achieve this degree of precision, three or more blood triglyceride measurements will be necessary, assuming typical analytical and biological variabilities of 3% and 21%, respectively (Figure 3). Ensuring that the patient has fasted before the test and that uniform patient preparation, blood-drawing procedures, and specimen handling are maintained may also be helpful in minimizing the large biological variability present in these measurements (Table 1) and thereby in limiting the number of consecutive blood specimens necessary to evaluate a response to these interventions.

In summary, values for blood triglyceride are not used to classify CHD risk. Because the biological variability of blood triglyceride relative to the analytical variability is high, increasing the analytical precision of the test will not improve test performance notably. Drugs used to treat high triglyceride concentrations frequently decrease values for blood triglyceride by >25%; to reliably detect differences of this magnitude, a CVb of <12.5% would be preferable. Because of the high biological variability, the CVb usually exceeds 20% (Figure 1). Multiple samplings, or minimizing biological variability, or both, are therefore necessary to reduce total test variability.

![Fig. 3. Effect of repeated measurements to reduce blood triglyceride variability](image)

**Symbols as in Fig. 2**

HDL-Cholesterol

**Importance.** A strong inverse relationship exists between HDL-cholesterol concentrations and CHD risk (23–25). Increases in HDL-cholesterol were correlated with a reduction in CHD endpoints (mortality) in the Helsinki Heart Study (15, 26), and have been associated with stabilization and regression of atherosclerosis as determined by sequential angiography (17). Because HDL-cholesterol is a strong independent predictor of CHD risk, its accurate measurement is essential before intervention with diet or drug therapy (2). Although the NCEP has yet to recommend specific intervention to increase HDL-cholesterol concentrations if they are initially low, consideration to increase low HDL-cholesterol concentrations has been suggested if the situation is associated with undesirable values for blood triglyceride or LDL-cholesterol (27). A major multicenter trial is currently under way to determine whether increasing HDL-cholesterol in subjects with normal values for triglyceride and LDL-cholesterol will reduce CHD endpoints (28).

Unlike interventions affecting LDL-cholesterol, diet and drug therapy are usually associated with only small changes in HDL-cholesterol concentrations. Therapy with gemfibrozil produced a 10% increase in HDL-cholesterol in the Helsinki Heart Study (15). Gemfibrozil and lovastatin were recently compared in normolipidemic men with subnormal values for HDL-cholesterol, and neither drug increased HDL-cholesterol by >12% (29). Niacin has been associated with somewhat larger increases in HDL-cholesterol, ranging from 13% to 31%, but frequently is poorly tolerated, owing to
severe flushing (30). Therapy with estrogen will also increase HDL, but the increases usually are also in the range of 10% to 15% (31).

Small changes in HDL-cholesterol may be clinically important. The Framingham risk equation (32), which accurately predicts the reduction in CHD risk obtained in intervention studies (33), can be used to compare the impact of changes in HDL on expected CHD incidence. For a nonhypertensive, non diabetic 60-year-old man with an LDL-cholesterol of 4.65 mmol/L (180 mg/dL), an HDL-cholesterol of 0.80 mmol/L (30 mg/dL), and a triglyceride value of 1.70 mmol/L (150 mg/dL), the likelihood of developing CHD in the next 10 years is 21%. Figure 4 illustrates the relative changes in CHD risk that could be expected by modifying HDL-cholesterol concentrations. A relatively large (40%) change in HDL risk occurs when HDL-cholesterol is modified by only 0.25 mmol/L (10 mg/dL) in either direction. For this reason, small changes in HDL-cholesterol produced by diet or drug therapy have clinical relevance to the clinician. The reliable detection of changes in HDL-cholesterol of 0.15 mmol/L (5 mg/dL) or less may be an important characteristic of a clinically useful assay.

**Variability.** The imprecision of HDL-cholesterol measurements and their potential impact on clinical decision-making have been reviewed (34). HDL-cholesterol measurements require two separate manipulations: the isolation of the HDL-containing fraction from plasma or serum, and then the measurement of cholesterol in this fraction. The former step appears to be associated with much of the error in precision (35). Interlaboratory analytical variability ranging from 9% to 38% has been reported (36). The CAP Comprehensive Chemistry Survey showed large interlaboratory coefficients of variation for HDL cholesterol, ranging from 11% to 16%, regardless of the precipitation technique used (7). This may be improving, although in the Canadian interlaboratory proficiency study, almost a third of the laboratories tested were more than ±10% from the target HDL-cholesterol values (37). Some clinical laboratories have successfully reduced the within-laboratory analytical variability for HDL-cholesterol to 5% or less (20), although test precision at many laboratories may be considerably higher (34). Although office-type analyzers for total cholesterol have achieved accuracy and precision standards rivaling those of the clinical laboratory, these techniques for HDL-cholesterol have been associated with unacceptable degrees of imprecision (38). As has been pointed out, the extent of deviation from acceptable performance in most routine clinical laboratories is unknown, because participation in quality-control surveys is usually voluntary (34).

Figure 5 shows the clinical impact of the large analytical variability of HDL-cholesterol measurements. For an analytical variability of 8%, the total test variability will be 10% (assuming biological variability of 5.8%, as reported by Mogadam et al. (20)); two-thirds of the total variability is ascribable to the poor test precision. A CV this large will make difficult the reliable detection of HDL-cholesterol responses to many diet and drug interventions. In addition, the accurate assessment of CHD risk may be impaired. Decreasing the CV from <5% would be necessary to detect a true difference between baseline and repeat HDL-cholesterol values of 10% with >95% confidence. To decrease the CV to this extent, either four separate blood specimens drawn on different dates, or three specimens, each performed in triplicate, would be required (Figure 5).

The right-hand side of Figure 5 shows results from an HDL assay with improved analytical precision (analytical CV = 3%). With this improved test, only 21% of total test variability is ascribable to analytical variability. Because the analytical imprecision is already low, performing the assay in duplicate or triplicate confers little benefit. However, the total test variability has been decreased to <7% with one specimen, and only two specimens are necessary to decrease it to <5%. Improving analytical precision in this case does reduce the total test variability, limiting the need for multiple specimens.

In summary, a significant component of variability of HDL-cholesterol measurements is due to the analytical imprecision of the test. Because HDL-cholesterol is a strong inverse predictor of CHD risk, and because most drug and diet interventions produce only small increases (≤15%) in HDL-cholesterol, the ability to reliably detect small changes in HDL-cholesterol is desirable. Improving the precision of the test will improve the clinician’s ability to accurately assess CHD risk, and will also allow a more reliable assessment of response to diet and drug therapy.

**LDL-Cholesterol**

*Importance.* LDL-cholesterol concentrations are important determinants of CHD risk. Multiple intervention trials demonstrate that lowering cholesterol,
whether by diet or drug therapy, decreases the incidence of CHD. These findings have been consistent with the rule of thumb that diminishing LDL-cholesterol by 1% decreases the incidence of CHD by 2% (39). NCEP guidelines stress the importance of setting individual goal values for LDL-cholesterol, and of utilizing both diet and drug therapy, if necessary, to achieve these goals. Therefore, determination of LDL-cholesterol is an essential part of dyslipidemia evaluation, and LDL-cholesterol determinations are necessary to assess response to therapy (2).

Therapy by diet modification in the compliant patient can produce decreases in LDL-cholesterol in the range of 10% to 15% (40). Psyllium reportedly lowers LDL-cholesterol by 5% to 15% (41, 42). Inhibitors of hydroxymethylglutaryl-CoA reductase, the drug therapy that most effectively lowers LDL-cholesterol, can decrease values by 30% or more (43). Niacin and bile acid sequestrants, the two first-line agents in therapy of hypercholesterolemia, usually decrease LDL-cholesterol by 15% to 25% (16, 44, 45). Because of the strong association between LDL-cholesterol and CHD risk, and because important interventions, such as diet therapy, may decrease LDL-cholesterol by 10% or less, reliable detection of small changes in LDL-cholesterol concentrations is necessary. To reliably detect a difference of this magnitude, a reduction of the total test variability to <5% would be desirable.

Variability. LDL-cholesterol is usually estimated by use of the Friedewald equation, which assumes that the amount of cholesterol in VLDL can be estimated by dividing the blood triglyceride concentration by five (10). If blood triglyceride values are <4.52 mmol/L (400 mg/dL), results by use of the Friedewald equation correlate well with LDL-cholesterol concentrations as determined by ultracentrifugation (46–48). However, several difficulties are associated with this calculation. First, blood triglyceride values >4.52 mmol/L (400 mg/dL) do not accurately predict VLDL-cholesterol concentrations; therefore, LDL-cholesterol cannot be estimated in the setting of hypertriglyceridemia. Second, the reliability of the LDL calculation depends on the accurate measurement of total-cholesterol, HDL-cholesterol, and triglyceride concentrations. Poor analytical precision in any or all of these measurements will contribute to the variability observed in values for LDL-cholesterol. Finally, the biological variability inherent in each of these three measurements will also contribute to the total variability of the LDL cholesterol value. The high biological variability of blood triglyceride, in particular, may interfere with the reliability of the LDL-cholesterol calculation.

In laboratories with good precision for total-cholesterol (CV ≤3%), HDL-cholesterol (<5%), and triglyceride (<3%) assays, the analytical variability for LDL-cholesterol as calculated by the Friedewald formula has been reported as <5%, with a biological variability of ~6% to 10% (19, 20). These components of variability contribute to a total test variability of ~11% (Figure 6). To decrease the CV to <5%, evaluation of five blood specimens is necessary (Figure 6A). Because the high biological variability contributes 80% of the total test variability, replicate measurements of total cholesterol, HDL-cholesterol, and triglyceride to minimize analytical variability offer little additional benefit.

To overcome these limitations of the Friedewald equation, direct measurements of LDL-cholesterol are being developed: selective precipitation of LDL apolipoprotein...
(apo) B and then quantification of the cholesterol co-
precipitated with the apo B. Several different precipitation
 techniques have been evaluated, including precipitation
 with heparin (49), polyvinyl sulfate (50), and dextran
 sulfate (51). The accuracy of these methods has been
 compared with those of standard ultracentrifugal tech-
niques and of the Friedewald equation, and the results
 have recently been reviewed (52, 53). Results obtained
 by use of the Friedewald formula agreed better with
 those by ultracentrifugation than did those by any of the
 precipitation methods. Further, all three methods
 proved less reliable in the setting of hypertriglyceri-
demia, and thus failed to overcome a central limitation
 of the Friedewald equation.

 Figure 6B demonstrates the potential advantages of
 using an improved method of LDL separation to mea-
sure LDL-cholesterol directly. Direct measurement of
 LDL-cholesterol offers the potential to improve both
 analytical and biological variability, because the preci-
sion of the LDL measurement would not depend upon
 the analytical and biological variability present in mea-
surements of triglyceride, total-, and HDL-cholesterol.
 In this hypothetical example, the analytical variability
 is decreased to 3%, the biological variability to 5%. The
 total test variability of one measurement is decreased to
 6%, and only two measurements are required to reduce
 it to <5% (Figure 6).

 In summary, LDL-cholesterol assay plays a central
 role in the evaluation and management of hypercholes-
terolemia. Detecting small differences in LDL-choles-
terol is clinically important, both to quantify changes in
 cardiovascular risk and to assess response to diet and
 drug therapy. The current approach to estimating LDL-
 cholesterol, the Friedewald calculation, is limited by the
 imprecision and biological variability of the three other
 lipid measurements. Improved direct approaches to
 LDL-cholesterol measurement are needed to overcome
 these limitations and allow a simple, reliable, and
 precise assessment of CHD risk.

 Conclusion

 Lipid measurements are associated with substantial
 test variability. To treat lipid disorders, the clinician
 must fully understand the implications of this variabil-
 ity. For example, if the expected effect of the interven-
tion is small relative to the CV, then multiple sam-
plings may be necessary if a true response to the
 treatment is to be reliably detected.

 To determine whether improvements in test perfor-
 mance will improve the clinical usefulness of the test,
 the clinician must also grasp the relationship between
 analytical and biological test variability. Improving the
 precision of the test will be clinically useful only if
 analytical variability is high relative to biological vari-
 ability. Even then, this approach will be relevant to the
 clinician only if (a) further reductions in test variability
 allow a better assessment of clinically important differ-
ences in cardiovascular risk, or (b) it allows more precise
determination of clinically important responses to an
 intervention. Further improvements in HDL- and LDL-
 cholesterol measurements are necessary for both rea-
sons. On the other hand, improving the precision of the
 blood triglyceride assay will not be as useful clinically,
 and, in any case, cannot be achieved by reducing anal-
lytical variability alone. Because understanding the
 laboratory characteristics (including both analytical
 and biological variability) and the clinical relevance of a
 test are both crucial for an accurate assessment of test
 performance, close communication between clinician
 and clinical chemist is mandatory. Through this collab-
oration, a critical evaluation of the strengths and limitations of a test will allow specific improvements in test performance to optimize health care delivery.

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Discussion

Bradley Copeland: Dr. Schectman, I appreciate the directness of your clinical judgment relating to laboratories. My concept of clinicians includes what I call the innate wisdom of clinicians. You implied that your formula came from, I think, this source—innate wisdom. The only thing in this I would change is I would divide by 2.8 or 3 instead of by 2. Now, in your slides, the y-axis demonstrates total test variability. How do you determine total test variability?

Gordon Schectman: Total test variability was determined from the equation, $(CV)_T^2 = (CV_A)^2 + (CV_V)^2$, assuming that the biological and analytical variabilities were known. To determine the effect of replicate measurements, I used the simple approach of dividing the analytical test variability by the square root of the number of replications. For repeated sampling, the total test variability was divided by the square root of the number of blood samples collected.

Bradley Copeland: My rule of thumb is three times the standard deviation.

Neil Greenberg: I would like to address the case of high-density lipoprotein (HDL). You contrasted the ideal performance of 3% for the analytical variability with the current typical performance of 8%. It still seemed to me that, because of the large biological variability, your graphs indicated substantial incremental improvement in the value of the test by doing replicate samples from the same patient, even when the analytical variation was 3%. Therefore, my question is, why would we even bother investing in improvements in the analytical variability if you still need to do multiple sampling on the apitents to get the sensitivity down to where you could detect real changes of 3–5%, which seem to be quite significant in terms of the overall risk assessment?

Gordon Schectman: The answer, in my opinion, relies on the importance of the HDL cholesterol measurement, and the fact that even improvements that appear to be small may be clinically important if you need to repeat the test less often. Small improvements in the total test variability for HDL due to improvements in analytical precision will make clinical decision-making easier as we try to assess small differences from baseline. As you say, you cannot overcome biological variability by improving analytical variability; for HDL, however, reducing the analytical test variability will provide an improvement in total test variability that will, in my opinion, translate into clinical benefit.

Calum Fraser: Cholesterol and lipids provide an interesting example of the generation of performance standards. The biological variability of cholesterol, triglycerides, etc., was all published many years ago. The messages about the significance of changes, numbers of specimens required, and so forth were all quite widely disseminated in the literature of clinical chemistry. However, after the NCEP guidelines were issued, it was very interesting to see a flurry of clinical interest and papers on biological variability that did not cite the literature of clinical chemistry but appeared in the medical journals along with editorials that merely restated what clinical chemists had been saying for years. This provides the interesting message that, if clinicians can provide guidelines and algorithms, the data on biological and analytical variability are available, and the impact of performance on clinical decision-making and desirable standards of performance can easily be calculated.

For calculated low-density lipoprotein cholesterol, it is important that we consider the biological variability of the calculated variable and then work back and assess the imprecision and inaccuracy required for the components that make up the variable. Simply to assume that triglycerides are very poorly controlled homeostatically and therefore imprecision of 10% is satisfactory is nonsense. The result is used to calculate another result.