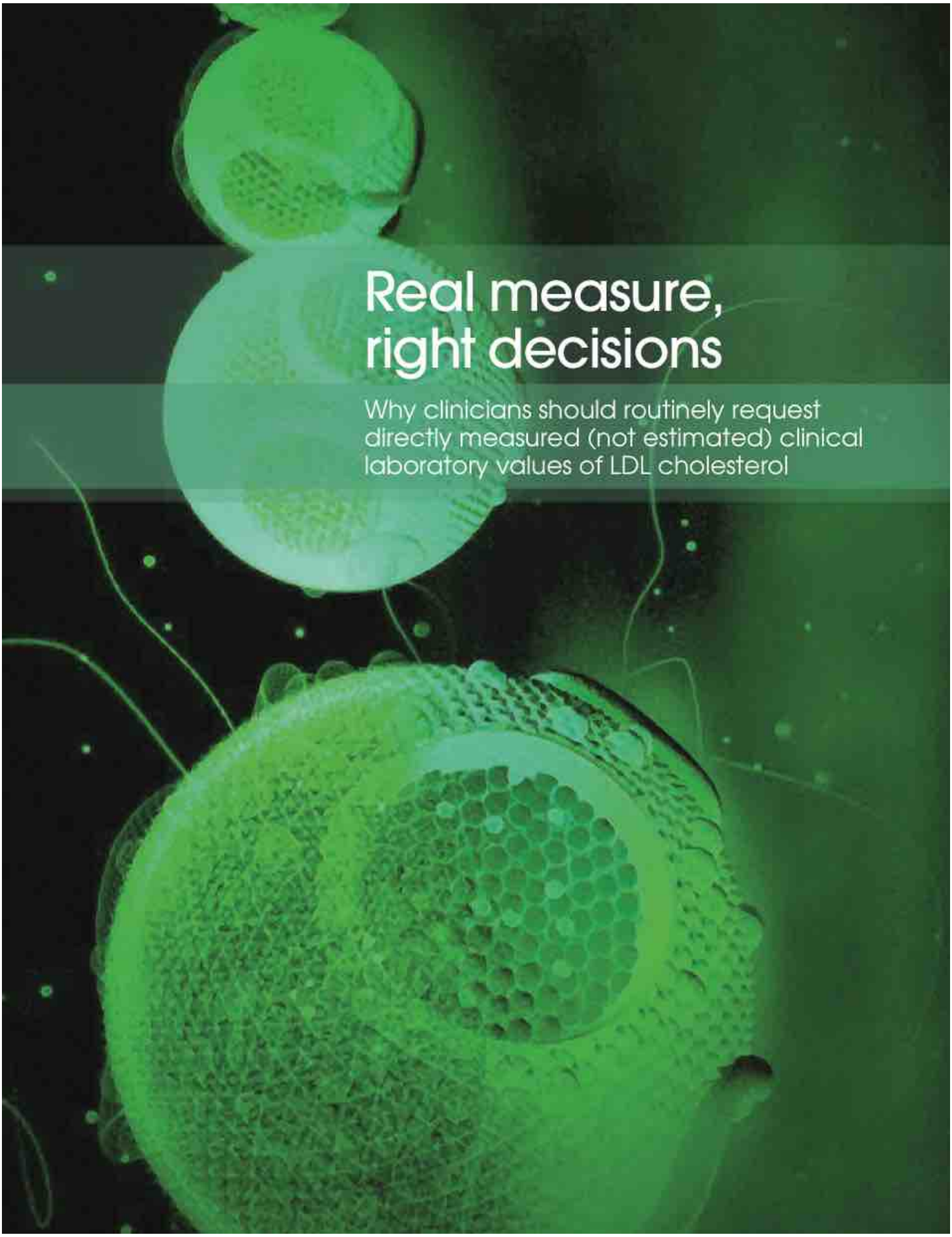

Real measure, right decisions

Why clinicians should routinely request directly measured (not estimated) clinical laboratory values of LDL cholesterol

LDL cholesterol levels: Not measured but estimated

The direct relationship between levels of low-density lipoprotein (LDL) cholesterol and the pathogenesis of atherosclerotic coronary vascular disease (CVD) has been well established, and CVD risk-reduction guidelines worldwide have consistently emphasized the importance of accurately measuring — and then if necessary lowering — levels of LDL cholesterol to prevent the development of CVD or to lessen the risk of cardiovascular events in persons with established CVD.[1-6]

It thus comes as something of a shock to learn that most LDL cholesterol values typically provided to clinicians for screening or managing their patients are not based on direct measurement of the low-density class of lipoprotein particles. Rather, most clinical decisions are based on LDL cholesterol values that are simply estimates.

The formula used to estimate most LDL cholesterol values is the Friedewald formula. In this formula, estimated very-low-density lipoprotein (VLDL) cholesterol (derived by dividing measured triglycerides by 5) and measured high-density lipoprotein (HDL) cholesterol are subtracted from measured total cholesterol (**Figure 1**).[7] To perform an estimate of LDL cholesterol with the Friedewald formula thus requires another estimate (VLDC cholesterol) plus three direct measurements (triglycerides, HDL cholesterol, and total cholesterol).

Given the unique importance of LDL cholesterol, it is ironic that LDL-cholesterol values used to initiate and monitor treatment are not based on direct measurement of the low-density class of lipoprotein particles. Despite the documented shortcomings of the Friedewald formula, up to 60% of clinical laboratories report estimated, not directly measured, LDL cholesterol values.[8]

The long-established gold standard for accurate measurement of LDL cholesterol is a specialty procedure called beta quantification. However, that technique is not available in most clinical laboratories. Beta quantification is a multistep procedure that combines ultracentrifugation (to separate particles on the basis of density) and chemical precipitation with quantification of lipoprotein fractions by chemical analysis of cholesterol. The procedure is regarded by the Centers for Disease Control and Prevention (CDC) as well as by the National Cholesterol Education Program (NCEP) of the National Heart, Lung, and Blood Institute (NHLBI) as the reference method for LDL cholesterol measurements.[8,9] However, beta quantification requires special equipment and is technique sensitive and labor intensive. When LDL cholesterol measurements are ordered, many clinical laborato-

LDL = TC – HDL – estimated VLDL
VLDL = TG ÷ 5

HDL = Measured high-density lipoprotein cholesterol
LDL = Estimated low-density lipoprotein cholesterol
TC = Measured total cholesterol
TG = Measured triglycerides
VLDL = Estimated very-low-density lipoprotein cholesterol

Figure 1. The Friedewald formula used to estimate LDL cholesterol values. This estimate of LDL cholesterol values — based on directly measured levels of total cholesterol, HDL cholesterol, and triglycerides, and on an estimate of VLDL cholesterol — is commonly reported instead of directly measured values.

ries simply estimate LDL cholesterol values using the Friedewald formula. Consequently, compared with values of total cholesterol, HDL cholesterol, and triglycerides, LDL cholesterol values have historically been recognized as the least reliable.[8]

Proposed in 1972, the Friedewald formula specified an approach to estimating VLDL cholesterol. Friedewald et al observed a 5:1 ratio between levels of triglycerides and levels of VLDL cholesterol.[7] They thus concluded that levels of VLDL cholesterol can be estimated by dividing measured levels of triglycerides by 5 (**Figure 1**). From the beginning, the Friedewald formula was recognized as invalid when triglyceride levels were >400 mg/dL because dividing high triglyceride levels by 5 would overestimate VLDL cholesterol, which in turn, in the Friedewald formula, would underestimate LDL cholesterol. But research has amply shown that even at triglyceride levels <400 mg/dL, LDL cholesterol values estimated by the Friedewald formula can be inaccurate.[10]

The use of the Friedewald formula to estimate LDL cholesterol values should be a concern to any clinician interested in accurate measurement and values. A Friedewald formula LDL cholesterol value not only includes an estimate of VLDL cholesterol, but it also includes the sum of the independent errors of the direct measurements of three different classes of lipoproteins. According to the Lipoprotein Measurement Working Group of the NCEP, even if the direct measurements of these three lipoprotein classes were within the limits of acceptable total-error performance, that would not guarantee that an estimate of LDL cholesterol could meet the nationwide total-error performance goal for LDL cholesterol established by the NCEP.[9]

Practical problems with the Friedewald formula

1. The need for fasting samples

Triglyceride concentrations increase after meals. Because transient postprandial triglyceride increases can significantly affect LDL cholesterol values estimated by the Friedewald formula, the NCEP Lipoprotein Measurement Working Group has recommended that the Friedewald formula only be applied to 12-hour fasting samples.[9]

In a study of lipoprotein concentrations in fed and fasted human volunteers, Cohn et al observed that because of increased triglyceride levels after a fat-rich meal, the Friedewald formula significantly overestimated VLDL cholesterol values ($p < 0.01$) and significantly underestimated LDL cholesterol values ($p < 0.01$) compared with direct measurements by beta quantification.[11] Even at 9 hours after a meal, VLDL cholesterol values were 50% higher ($p < 0.01$) and LDL cholesterol values were 8% lower ($p < 0.01$) than values directly measured by beta quantification.

Clinicians certainly cannot tell the difference between patients who fast for 12 hours before a blood sample is drawn and those who do not. But the consequence for patients who do not fast can be important: Values estimated by the Friedewald formula could be below actual values as a result of the postprandial triglyceride elevation. The NCEP Lipoprotein Measurement Working Group has recommended that all nonfasting Friedewald-formula LDL cholesterol values be discarded.

2. Patient populations with elevated levels of triglycerides

Triglyceride measurement is an important biomarker for CVD risk. According to the 2011 scientific statement of the American Heart Association (AHA) on triglycerides and CVD, mean triglyceride levels have been rising in the United States in concert with the epidemic of obesity, insulin

Table 1. Patient populations in which the use of the Friedewald formula for estimating LDL cholesterol values is problematic

Patients who might have difficulty complying with the 12-hour fasting requirement
Senior citizens • Adolescents and children • Patients with compromised immune systems
Patients with metabolic syndrome or diabetes mellitus • Patients on multiple medications
Patients with elevated triglyceride levels
Gender and ethnic subgroups known to have a tendency toward elevated triglyceride levels
Patients with secondary hypertriglyceridemia
Patients with specific medical conditions
Patients with diabetes mellitus • Patients with chronic end-stage renal disease
Patients with cirrhosis • Patients on hemodialysis

proteins in order to facilitate the removal of HDL and VLDL in the specimen. In 1997, the N-geneous LDL cholesterol test was introduced as the first automated homogeneous LDL cholesterol test in the United States. The term "homogeneous" describes an assay that can be fully automated in a single reaction vessel on a routine clinical chemistry analyzer, offering significant cost savings and ease of use to laboratories. The assay employs a homogeneous method with two liquid reagents to segregate LDL cholesterol.

As a fully automated reagent system, N-geneous LDL revolutionized routine LDL cholesterol testing, enabling laboratories to realize significant operational benefits while at the same time improving the quality of LDL cholesterol results. This assay offers significant cost savings and ease of use for laboratories. Other manufacturers have introduced homogeneous assays for directly measuring LDL cholesterol, each employing different homogeneous methods. All suppliers of these homogeneous direct LDL cholesterol assays provide reagent sets that contain two reagents and that are readily adaptable to clinical chemistry analyzers.

Performance goals for LDL cholesterol measurements, developed by the Lipoprotein Measurement Working Group of the NCEP, became available in 1995.[9] For tests of LDL cholesterol, like the N-geneous LDL cholesterol test, the following NCEP performance criteria apply[8]:

- **An inaccuracy value of ≤4%.** Inaccuracy refers to the systemic bias of a test in comparison with the reference measurement.
- **An imprecision value of ≤4%.** Imprecision refers to how closely repeated measurements agree with each other.
- **A total error of ≤12%.** Total error is the combination of the values for inaccuracy and imprecision. The total error criterion means that the values obtained by a clinical diagnostic test should be within 12% of the true values as determined by the reference method.

The N-geneous LDL cholesterol test has met each of these NCEP performance goals for LDL cholesterol testing. For regulatory performance data on the N-geneous LDL cholesterol test, including a range of 12 different studies, please see the monograph ***N-geneous LDL Cholesterol Test: The Science Behind the First Commercially Developed Homogeneous LDL Cholesterol Test.*** In addition, the studies of the N-geneous LDL cholesterol test referenced below were independently conducted and reported in the peer-review literature.

Rifai et al evaluated the total-error performance of the N-geneous LDL cholesterol test in comparison with the Friedewald formula in 199 fasting samples, using the beta-quantification reference method.[20] The total-error performance of the N-geneous LDL cholesterol test was 6.75%, while that of the Friedewald formula was 11.6%. The investigators also found that LDL cholesterol values measured by the N-geneous LDL cholesterol test were not significantly affected by increased triglyceride concentrations up to 1078 mg/dL.

1. The N-geneous LDL cholesterol test and fasting samples

One advantage of direct measurement of LDL cholesterol compared with an estimate by means of the Friedewald formula is that the direct test can be applied to both fed and fasting blood samples. In another analysis, Rifai et al applied the N-geneous LDL cholesterol test to 36 fasting samples from volunteers and then, on the same day, applied the test to a second set of 36 samples after the same volunteers had consumed a high-fat fast-food meal of a sausage-and-egg sandwich and hash browns.[20] Although the triglyceride levels of the patients increased after the meal, there was no statistically significant difference between the LDL cholesterol values measured by the N-geneous LDL cholesterol test before and after the meal.

2. The N-geneous LDL cholesterol test and special patient populations

Ragland et al evaluated the accuracy of LDL cholesterol values in patients with controlled diabetes mellitus as measured by either the N-geneous LDL cholesterol test or the Vertical Auto Profile (VAP) technique or as estimated by the Friedewald formula.[21] They found that the LDL cholesterol values measured by the N-geneous LDL cholesterol test had good correlation with values measured by the VAP technique (**Figure 4A and 4B**). The LDL cholesterol values measured by the N-geneous LDL cholesterol test also showed minimal bias throughout the range of triglyceride levels. In contrast, the LDL cholesterol values estimated by the Friedewald formula showed a prominent negative bias as triglyceride levels increased (**Figure 4B**). In fact, the investigators found no interference of

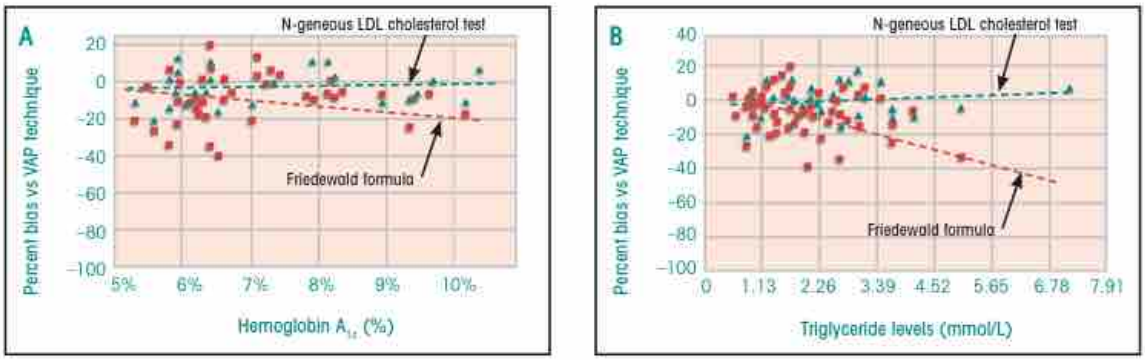

Figure 4. The bias of the LDL cholesterol values measured by the N-geneous LDL cholesterol test (green triangle symbols) and estimated by the Friedewald formula (red box symbols) versus the values determined by the Vertical Auto Profile (VAP) technique as affected by variations for 52 patients with controlled diabetes mellitus in measurement of (A) hemoglobin A_{1c} and (B) triglyceride levels. The LDL cholesterol values measured by the N-geneous LDL cholesterol test had good correlation with the values measured by the VAP technique, and they showed minimal bias throughout the range of triglyceride levels.[21]

Table 2. Intensity of statin therapy for the four statin benefit groups

	High-intensity statin therapy	Moderate-intensity statin therapy	Low-intensity statin therapy
Recommended amount of LDL cholesterol lowering by means of daily statin dose	≥50%	30% to 49%	<30%
Indications	Statin benefit group 1, patients <75 years Statin benefit group 2 Statin benefit group 3, for those persons with 10-year atherosclerotic CVD risk ≥7.5% Statin benefit group 4, for those persons with 10-year atherosclerotic CVD risk ≥7.5%	Statin benefit group 1, patients ≥75 years Statin benefit group 3 Statin benefit group 4, for those persons with 10-year atherosclerotic CVD risk ≥7.5% Statin benefit group 4, for those persons with 10-year atherosclerotic CVD risk between 5% and <7.5%	To be used when high or moderate intensity statin therapy cannot be tolerated

Statin benefit group 1: Persons with clinical atherosclerotic cardiovascular disease, defined as acute coronary syndromes or a history of myocardial infarction, stable angina, coronary or other arterial revascularization, stroke, transient ischemia attack, or peripheral arterial disease presumed to be of atherosclerotic origin.

Statin benefit group 2: Persons with LDL cholesterol ≥190 mg/dL.

Statin benefit group 3: Persons with diabetes aged 40 to 75 years and with an LDL cholesterol level between 70 mg/dL and 189 mg/dL who undergo an estimated 10-year atherosclerotic CVD risk using the risk calculator.

Statin benefit group 4: Persons aged 40 to 75 years without diabetes and with an LDL cholesterol level between 70 mg/dL and 189 mg/dL, who undergo an estimated 10-year atherosclerotic CVD risk using the risk calculator.

Source: Stone NJ et al.[24]

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resistance, and type 2 diabetes mellitus.[12,13] In the current triglyceride classification, levels between 150 mg/dL and 199 mg/dL are considered borderline high, levels between 200 mg/dL and 499 mg/dL are considered high, and levels ≥500 mg/dL are considered very high.

Figure 2 shows the prevalence of high triglyceride levels by age, gender, and ethnicity based on the cutpoints of ≥150 mg/dL and ≥200 mg/dL, with data derived from the US National Heart and Nutrition Examination Survey from 1999 to 2008.[13] Today, more than a third of adults ≥50 years of age have triglyceride levels ≥150 mg/dL. More than a third of all men have triglyceride levels ≥150 mg/dL, and 1 in 5 men — as well as 1 in 5 adults between 50 and 69 years of age — have triglyceride levels ≥200 mg/dL. The overall prevalence of triglyceride levels ≥500 mg/dL is between 1% and 2%; however, in some population subgroups, such as Mexican American men between 50 and 59 years of age, the prevalence is as high as 9%.

Again, when the Friedewald formula was developed, it was recognized as being invalid for patients with triglycerides levels ≥400 mg/dL — the formula would overestimate VLDL cholesterol values and thus underestimate LDL cholesterol values. Today, it is known that the reliability of the Friedewald formula is decreased even for "borderline high" levels of triglycerides. McNamara et al compared LDL cholesterol values estimated by the Friedewald formula with beta-quantification measurements in 4797 normal and dyslipidemic fasting adults. The investigators found that estimated LDL cholesterol values were not within ±10% of directly measured LDL cholesterol values for 16% of patients with triglyceride levels <200 mg/dL, for 23% of patients with triglyceride levels between 201 mg/dL and 300 mg/dL, for 41% of patients with triglyceride levels between 301 mg/dL and 400 mg/dL, and for 59% of patients with triglyceride levels between 401 mg/dL and 600 mg/dL (**Figure 3**).[10]

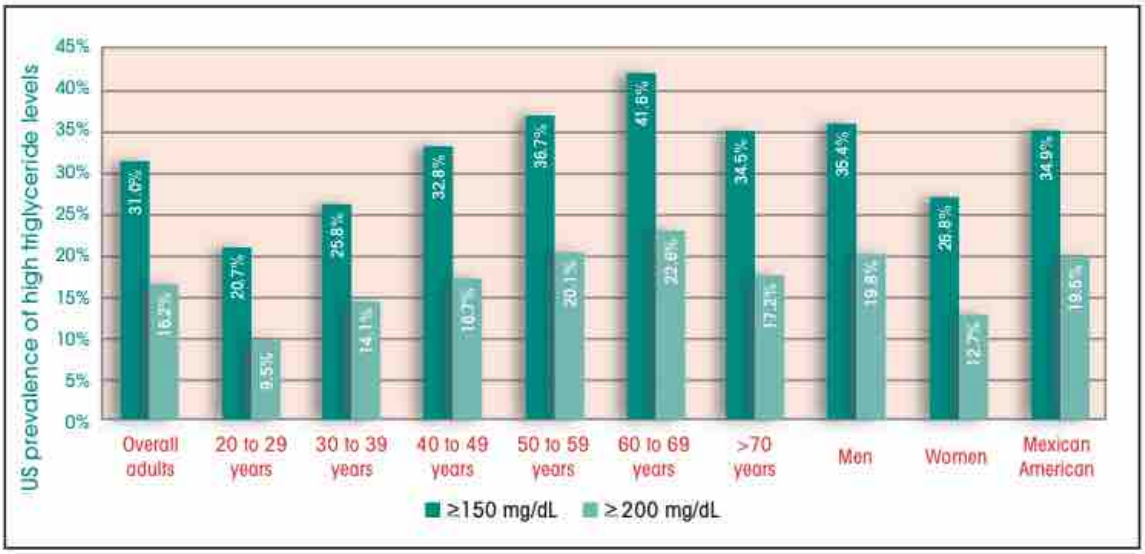

Figure 2. The overall prevalence of high triglyceride levels in the United States at the cutpoints of ≥150 mg/dL and ≥200 mg/dL. Source: National Health and Nutritional Examination Survey, 1999–2008, as reported in the 2011 Scientific Statement on triglycerides and cardiovascular disease from the American Heart Association.[13]

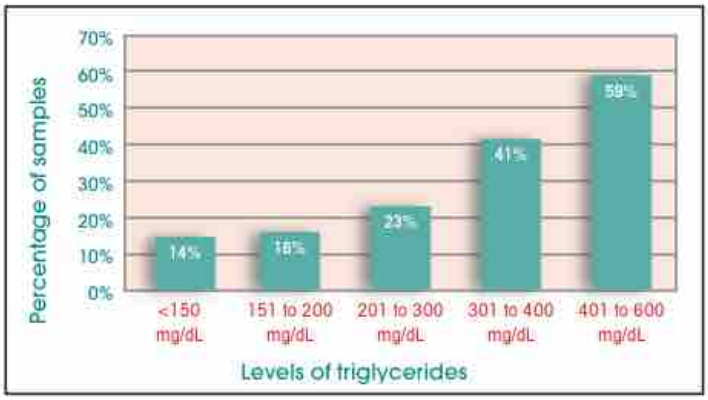

Figure 3. The percentage of 4797 normal and dyslipidemic adults at different ranges of triglyceride levels in whom LDL cholesterol values estimated by the Friedewald formula were not within 10% of LDL cholesterol values measured by the beta-quantification reference method. Adapted from McNamara et al.[10]

3. Specific patient populations for whom the use of the Friedewald formula is problematic

Because diabetes mellitus is associated with elevated triglyceride levels, patients with diabetes mellitus form a huge subgroup of patients for whom LDL cholesterol values estimated by the Friedewald formula are not always accurate. Patients with diabetes mellitus also have problems fasting for 12 hours and are susceptible to large postprandial triglyceride elevations.

In an assessment of the accuracy of LDL cholesterol values for patients with controlled type 1 and type 2 diabetes mellitus, Rubies-Prat et al found that LDL cholesterol values estimated by the Friedewald formula were within 10% of LDL cholesterol values measured by the beta-quantification reference method only 49% of the time.[14] The Friedewald formula overestimated LDL cholesterol values in diabetes patients 39% of the time and underestimated LDL cholesterol values 13% of the time. The mean differences between the estimated Friedewald-formula and measured beta-quantification LDL cholesterol values were statistically significant for type 2 diabetic patients. Similarly, Sibal et al compared LDL cholesterol values estimated by the Friedewald formula with beta-quantification values in patients with controlled type 1 diabetes mellitus.[15] They concluded that estimated LDL cholesterol values are "unsuitable" for use in these patients for achieving therapeutic targets.

The Friedewald formula has also been found inadequate for patients with end-stage renal disease[16] and with hepatic failure.[17] It cannot be used for patients with the rare condition of type III hyperlipoproteinemia.[18]

Given the practical problems with the application of the Friedewald formula (the need for fasting samples, the avoidance of patient populations with elevated triglyceride levels) (**Table 1,** page 6), the NCEP Lipoprotein Measurement Working Group in 1995 called for the development of "new methods for LDL measurement . . . capable of quantifying LDL cholesterol directly . . . not based on calculations of difference between two or more measured values."[19]

That call has been answered by the N-geneous LDL cholesterol test.

The N-geneous LDL cholesterol test

The first available direct LDL cholesterol test was the Direct LDL Cholesterol Immunoseparation Reagent (Sekisui Diagnostics), which used affinity-purified goat polyclonal antisera to specific human apolipo-

high triglyceride levels with LDL cholesterol values as measured by the N-geneous LDL cholesterol test — even at triglyceride levels as high as 1245 mg/dL. The N-geneous LDL cholesterol test displayed good linearity for LDL cholesterol values between 30 mg/dL and 240 mg/dL, with excellent within-run and between-run precision. Overall, the presence of controlled diabetes mellitus did not affect the performance of the N-geneous LDL cholesterol test.

Bairaktari et al evaluated the N-geneous LDL cholesterol test and four other methods (including two different versions of the Friedewald formula) in comparison with the beta-quantification reference method for measuring LDL cholesterol in 98 patients on hemodialysis.[22] Patients on hemodialysis commonly exhibit quantitative lipoprotein abnormalities, such as hypertriglyceridemia and low HDL-cholesterol levels, but also qualitative lipoprotein abnormalities, which can interfere with routine laboratory measurement. The investigators reported that compared with the reference method, the N-geneous LDL cholesterol test had the least bias of any of the alternative methods, and offered "an improved approach" to LDL cholesterol analysis in patients on hemodialysis.

Assay ordering and reimbursement

Currently, the 2014 Centers for Medicare and Medicaid Services (CMS) reimbursement for the N-geneous LDL cholesterol test is $13.02. In contrast, the 2014 CMS reimbursement for a lipid panel — which includes the Friedewald formula estimate of LDL cholesterol — is $18.27.[23] The CPT code for a direct measurement of LDL is 83721.

According to the 2014 CMS Medicare National Coverage Determinations Manual, for monitoring dietary or pharmacologic therapy of LDL cholesterol in the first year after initiation of such therapy, a direct measure of LDL cholesterol or the cholesterol panel may be ordered up to six times. The direct measure or the panel can even be ordered more frequently during that first year after initiation of therapy "for marked elevations or for changes to anti-lipid therapy due to inadequate initial patient response to dietary or pharmacologic therapy." After treatment goals have been achieved, the direct test or the lipid panel can be ordered three times yearly.

Providers must select the most appropriate test for their patients. Clinicians need to regularly specify that a direct LDL cholesterol test be used when sending patient samples for laboratory processing. Most laboratory forms will have a provision for ordering a direct measurement of LDL cholesterol.

Medical benefits of direct LDL cholesterol measurement

With direct measurement of LDL cholesterol, both clinicians and patients can have increased confidence about the accuracy of LDL cholesterol values. An unexpected rise in the LDL cholesterol value at a regular follow-up visit can cause a great deal of concern to both patient and clinician alike.

Moreover, because direct measurement of LDL cholesterol provides accurate and precise values with nonfasting samples, it also improves the level of convenience for clinicians and patients. Blood samples can be drawn at any time, under any circumstances, for measurement of LDL cholesterol values, without the requirement that patients previously fast for 12 hours. Clinicians can be confident

that the health status of their patients — for example, whether or not they have diabetes mellitus or might be in a subgroup with high triglyceride levels — will not affect the quality of the LDL cholesterol values that derive from testing.

In the 2002 report of the Adult Treatment Panel of the NCEP, a program overseen by the US National Heart, Lung and Blood Institute (NHLBI), the recommended LDL cholesterol therapeutic goal for adults was a value <100 mg/dL.[2] For high-risk persons, the recommended LDL cholesterol goal was either a value <70 mg/dL or a total percentage reduction of LDL cholesterol in the range of 30% to 40%.

In November 2013, the American College of Cardiology (ACC) and the AHA, taking up the guideline mantle from the NHLBI, issued new recommendations for reducing blood cholesterol to address the risk of atherosclerotic CVD in adults.[1] Emphasizing evidence from randomized controlled trials that the incidence of CVD events is most affected by the intensity of statin use, the new ACC/AHA guideline shifted the focus from specific LDL cholesterol treatment targets — such as <100 mg/dL or <70 mg/dL — to the appropriate intensity of statin use, gauged by overall percentage reductions of LDL cholesterol values. There are three categories of intensity of statin use in the new ACC/AHA guidelines: "high intensity," which can lower baseline LDL cholesterol values by >50%; "moderate intensity," which can lower baseline LDL cholesterol values by 30% to 49%; and "low intensity," reserved for people who cannot tolerate "high intensity" or "moderate intensity" statin use.

The new guidelines distinguish four patient categories and indicate for each the appropriate amount of LDL cholesterol lowering to be achieved with different intensities of statin therapy. The four patient categories are: (1) individuals with clinical atherosclerotic CVD; (2) individuals with LDL cholesterol values ≥190 mg/dL; (3) individuals aged 40 to 75 years with diabetes, with LDL cholesterol values between 70 mg/dL and 189 mg/dL, but without atherosclerotic CVD, for whom 10-year atherosclerotic CVD risk has been estimated using the guideline risk calculator (available at http://my.americanheart.org/cvriskcalculator); and (4) individuals aged 40 to 75 years without diabetes, with LDL cholesterol values between 70 mg/dL and 189 mg/dL, for whom 10-year atherosclerotic CVD risk has been estimated using the risk calculator. **Table 2** (page 10) breaks out the appropriate intensity of statin therapy for each of these patient categories, and for some subgroups within the categories, based on the guidelines.

Real measure, right decision

In the primary and secondary prevention of CVD — for which an entire decision structure has been elaborated on the basis of obtaining regular accurate LDL cholesterol values for either exact or percentage-reduction therapeutic targets — direct measurement of LDL cholesterol can make the difference between optimal and suboptimal treatment decisions.

An estimate calculated by means of the Friedewald formula should no longer be accepted by clinicians as the only option for obtaining LDL cholesterol values. The mechanisms are in place for ordering and obtaining reimbursement for direct measurement of LDL cholesterol with the N-geneous LDL cholesterol test.

Because LDL cholesterol measurement is so important, clinicians now have the option of insisting on the most accurate and most reliable test. For the right therapeutic decisions, the real measure — the direct measure — should be requested.

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