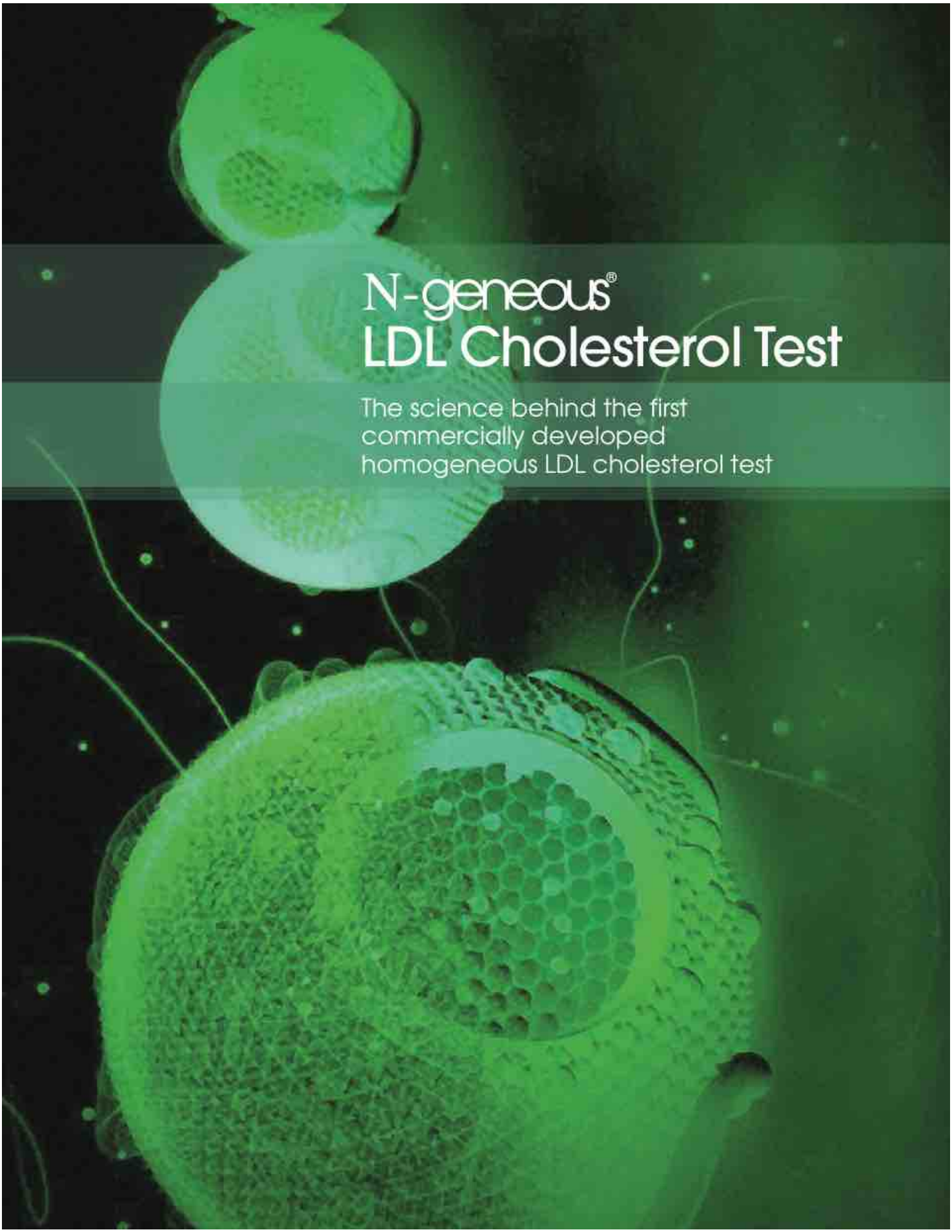
N-geneous®
LDL Cholesterol Test
The science behind the first
commercially developed
homogeneous LDL cholesterol test

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N-geneous® LDL Cholesterol Test

The science behind the first commercially developed homogeneous LDL cholesterol test

CONTENTS

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Performance data for the N-geneous LDL cholesterol test

The following sections report performance studies for the N-geneous LDL test. The N-geneous LDL test was directly compared with the beta-quantification reference method in a study conducted at Pacific Biometrics Research Foundation (Seattle, Washington), a CRMLN reference laboratory. The N-geneous LDL test was also directly compared with the first-generation Direct LDL Cholesterol Immunoseparation test in a study conducted at Sekisui Diagnostics on a Hitachi 911 auto analyzer. Reported in the following pages are the 11 different studies that were conducted: method comparisons; precision; total error; linearity; interference; sample type; fast-fed; sample integrity; working reagent stability; reagent interchangeability; and calibrator stability.

METHOD COMPARISONS

Figure 3 depicts the regression equations and correlations that resulted when the N-geneous LDL test was compared to beta-quantification (using 54 normotriglyceridemic samples) and the Direct LDL immunoseparation test (using 92 normotriglyceridemic samples). In the comparison of the N-geneous LDL test versus beta-quantification, the mean bias was –2.62 mg/dL and the mean percentage bias was –1.9%. In the comparison of the N-geneous LDL test versus the Direct LDL immunoseparation test, the mean bias was –2.81 mg/dL, and the mean percentage bias was –2.0%. In this analysis, the N-geneous

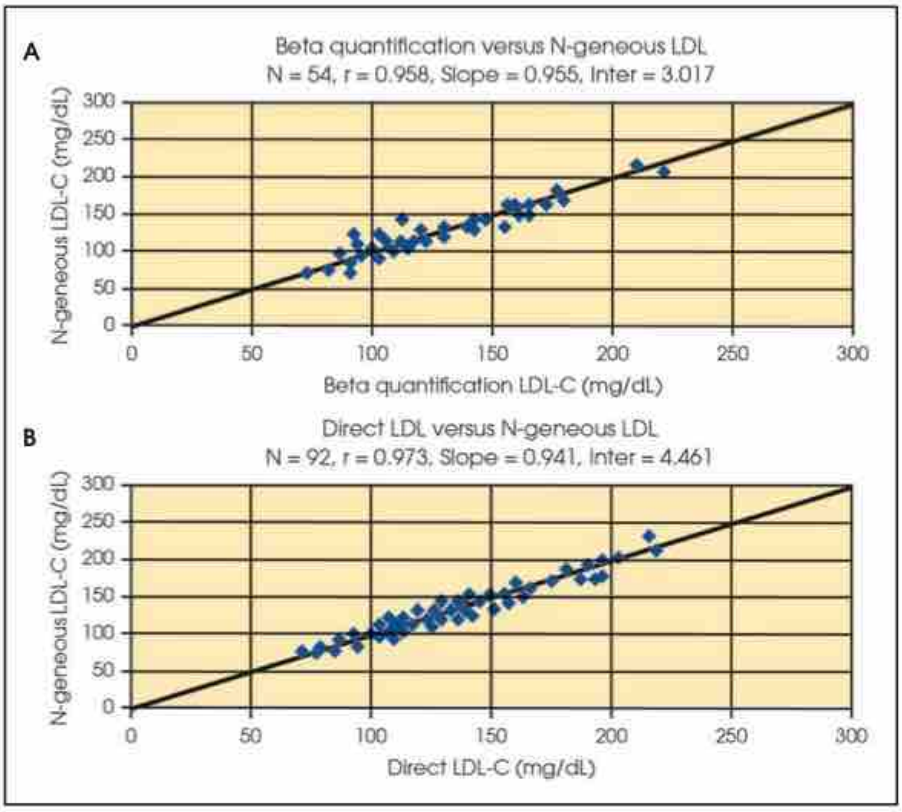


Figure 3. Regression equations and correlations with normotriglyceridemic specimens **(A)** for the N-geneous LDL test versus the beta-quantification reference method and **(B)** for the N-geneous LDL test versus the Direct LDL immunoseparation test.

THE BETA-QUANTIFICATION REFERENCE METHOD

The Centers for Disease Control (CDC) reference measurement procedure is the national standard for LDL cholesterol testing, as recommended in 1995 by the expert laboratory panel convened by the NCEP.[3,13] The CDC reference method uses a single ultracentrifugation of plasma at its own nonprotein density of 1.006 g/mL. The VLDL and chylomicrons float in the supernate after centrifugation, while the LDL and HDL remain below in the infranate. Cholesterol is measured in the infranate (for the sum of LDL and HDL cholesterol), and then the HDL cholesterol is precipitated and measured alone. LDL cholesterol is then calculated as the total infranate (LDL plus HDL) cholesterol minus the separately measured HDL cholesterol. This LDL reference procedure is called "beta quantification" because LDL lipoproteins migrate to the beta zone during serum protein electrophoresis — the movement of charged particles in a fluid or gel under the influence of an electric field. Beta quantification for LDL cholesterol is technically demanding and outside the range of normal clinical laboratories.

The expert laboratory panel convened by the NCEP in 1995 also established analytical performance goals for measurement of lipids and major lipoproteins based on the need to reliably categorize patients, and the CDC created a Cholesterol Reference Method Laboratory Network (CRMLN) that certifies clinical diagnostic products measuring TC, HDL cholesterol, and LDL cholesterol.[13] The CRMLN laboratories use the appropriate lipoprotein reference methods or designated comparison methods rigorously standardized to the CDC reference methods. For tests of LDL cholesterol, like the N-geneous LDL test, the following NCEP performance criteria apply[13]:

- **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 FRIEDEWALD FORMULA

Developed in 1972, the Friedewald formula calculates LDL cholesterol by using measured values of TC, HDL cholesterol, and TG, and an estimated value for VLDL cholesterol (VLDL cholesterol = TG/5).[1] The Friedewald formula used the following calculation:

LDC cholesterol = TC *minus* HDL cholesterol *minus* VLDL cholesterol*

*Estimation of VLDL cholesterol = TG/5

As can be seen, the formula requires three independent lipid analyses, each of which contributes a potential source of total error. The formula also involves a potentially inaccurate estimate of VLDL cholesterol, since no direct VLDL cholesterol assay is available. The divisor of 5 for the calculation of VLDL cholesterol (TG/5) in the Friedewald formula adds

CONTENTS *continued*

ABBREVIATIONS

ACC	American College of Cardiology
AHA	American Heart Association
CVD	Cardiovascular disease
CDC	Centers for Disease Control
CRMLN	Cholesterol Reference Method Laboratory Network
FDA	Food and Drug Administration
HDL	High-density lipoprotein
IDL	Intermediate-density lipoprotein
LDL	Low-density lipoprotein
NCEP	National Cholesterol Education Program
NHLBI	National Heart, Lung and Blood Institute
TC	Total cholesterol
TG	Triglycerides
VLDL	Very-low-density lipoprotein

Introduction

The N-geneous LDL cholesterol test (Sekisui Diagnostics), developed in the late 1990s, was the first homogeneous LDL cholesterol assay to be made commercially available worldwide. Today, the phrase "direct LDL cholesterol test" can refer to any one of several different homogeneous methods of directly measuring LDL cholesterol — methods that do not require either a preliminary separation step or manual manipulation of the sample for measuring LDL cholesterol values. The N-geneous LDL cholesterol test is one of those homogeneous methods.

Direct LDL cholesterol tests were specifically developed to overcome limitations of the Friedewald formula, a method of estimating LDL cholesterol values that is based on separate measurements of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and triglycerides (TG).[1-3] As determined by the Friedewald formula, calculated LDL cholesterol values represent the accumulated imprecision and inaccuracy of three different measurements, are not accurate in the presence of elevated triglyceride levels (such as in diabetic patients), and require fasting samples.[2] Given that LDL-cholesterol values have formed the cornerstone of cardiovascular disease management, it has been an ongoing irony of contemporary medicine that this estimate is used for treatment decisions rather than a real and direct measure, a measure that is both standardized and reproducible. In 1995, the expert laboratory panel of the US National Cholesterol Education Program (NCEP) stated that LDL cholesterol should be directly measured because of the limitations of the Friedewald formula.[3]

This monograph describes how the N-geneous LDL cholesterol test works and presents the original comparative research that earned approval of the test by the US Food and Drug Administration (FDA). The use of the N-geneous LDL cholesterol test is discussed in the context of the new 2013 guideline from the American College of Cardiology (ACC) and American Heart Association (AHA) on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular disease (CVD).[4]

LDL CHOLESTEROL

There is a demonstrated direct relationship between levels of LDL cholesterol and the pathogenesis of atherosclerotic CVD.[5] In recognition of this direct relationship, major CVD risk-reduction guidelines worldwide have strongly emphasized the importance of measuring — and then if necessary lowering — levels of LDL cholesterol in order to prevent the development of CVD or to lessen the risk of cardiovascular events in persons with established CVD.[4-9]

Substantial clinical trial evidence has demonstrated that a reduction of LDL cholesterol by means of drugs and/or lifestyle changes can lower cardiovascular events.[10] Consequently, for 40-plus years, a large ongoing public health effort has recommended that all adults be screened for cardiovascular risk by measurement of TC, LDL cholesterol,

HDL cholesterol, and TG. All four of these lipoprotein measurements can help assess adult CVD risk. But the consensus cornerstone for making treatment decisions has been the measurement or calculation of LDL cholesterol. In the 2003 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. 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 ACC and AHA, taking up the guideline role from the NHLBI, issued new recommendations for reducing blood cholesterol to reduce the risk of atherosclerotic CVD in adults. Noting that 1 in 3 Americans still die of heart disease and stroke, these recommendations again emphasized that most CVD is preventable with a healthy lifestyle and with treatment of high cholesterol and blood pressure.

However, the ACC/AHA guideline shifts the focus from specific LDL cholesterol treatment targets, such as <100 mg/dL or <70 mg/dL, to the appropriate intensity of statin therapy, which is gauged by the overall percentage reduction of LDL cholesterol values. The guideline authors noted three problems associated with the previous strategy of reducing LDL cholesterol levels below certain specific target goals. First, the evidence from randomized controlled trials did not consistently indicate what the target LDL cholesterol goals should be. Second, that evidence did not indicate the magnitude of additional CVD risk reduction that could actually be obtained by successfully targeting the goal of LDL cholesterol <70 mg/dL rather than <100 mg/dL. The third and most compelling reason for shifting the guideline focus was the accumulated wisdom that the evidence did not adequately take into account the potential adverse effects from the aggressive dose titration and/or multidrug therapy (involving the addition of niacin or other agents along with statins in the treatment regimen) expressly employed in order to achieve the target LDL cholesterol values.

The evidence from randomized controlled trials showed instead that the incidence of CVD events was affected by the intensity of statin use: "high intensity" use, which can lower baseline LDL cholesterol values by >50%, or "moderate intensity" use, which can lower baseline LDL cholesterol values by 30% to 49%. ("Low intensity" use is reserved for people who cannot tolerate "high intensity" or "moderate intensity" statin use).

The shift in emphasis in the new ACC/AHA guidelines thus represents a backing off from targeting specific LDL cholesterol values to a position of targeting percentage reductions of LDL cholesterol values. As guidance for circumstances when baseline levels of LDL cholesterol are not known, the guideline authors reference the finding in randomized clinical trials that achievement of LDL cholesterol values <100 mg/dL was associated with high-intensity statin therapy.[11]

The new guidelines distinguish four patient categories and indicate for each the appropriate amount of LDL-cholesterol lowering

to be achieved with 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; and (4) individuals aged 40 to 75 years of age 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 1** 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.

Table 1. Intensity of statin therapy for the four main statin benefit groups

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

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 ischemic 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.[11]

A primer on LDL cholesterol testing

LIPOPROTEINS

Plasma lipoproteins are spherical particles containing varying amounts of free cholesterol, cholesteryl esters, triglycerides, phospholipids, and proteins. They are classified by their buoyant density, which inversely reflects their size. The free cholesterol, phospholipids, and proteins constitute the outer surface of the lipoprotein particle, while the inner core of the particle contains esterified cholesterol and triglycerides. **Table 2** shows the five major classes of lipoproteins: chylomicrons, very-low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), LDL, and HDL.[12]

Lipoproteins enable the transportation of lipids — such as cholesterol, phospholipids, and triglycerides — within extracellular fluid, including the bloodstream. Of these different lipoproteins, LDL is the major carrier of cholesterol in plasma. LDL is defined as a lipoprotein particle with density ranges between 1.019 g/mL and 1.063 g/mL. In terms of percentage weight, the LDL particle contains approximately 50% cholesterol, 25% protein, 20% phospholipid, and 5% triglyceride.

The current methods of measuring LDL cholesterol can be broken down into three categories: (1) the LDL cholesterol reference method; (2) the calculation, called the Friedewald formula, that estimates LDL cholesterol values; and (3) the Direct LDL cholesterol method. Homogenous methods do not require either a preliminary separation step (like ultracentrifugation) or manual manipulation of the sample: the sample tube can be run directly on an automated chemistry analyzer.

Table 2. The five major classes of lipoproteins

	Chylomicrons	VLDL	IDL	LDL	HDL
Density (g/mL)	<0.95	<1.006	1.006–1.019	1.019–1.063	1.063–1.210
Diameter (nm)	75–1200	30–80	25–35	18–25	50–120
Mass (kDa)	400,000	10,000–80,000	5,000–10,000	2,300	175–360
% protein (surface)	1.5%–2.5%	5%–10%	15%–20%	20%–25%	40%–55%
% phospholipid (surface)	7%–9%	15%–20%	22%	15%–20%	20%–35%
% free cholesterol (surface)	1%–3%	5%–10%	8%	7%–10%	3%–4%
% triglycerides (core)	84%–89%	50%–65%	22%	7%–10%	3%–5%
% cholesteryl esters (core)	3%–5%	10%–15%	30%	35%–40%	12%

Adapted from: Hardikar W, Suchy FJ.[12]

error to all LDL cholesterol estimates, but it is especially inappropriate for individuals with elevated triglyceride levels.

The drawbacks of using the Friedewald formula for calculation of LDL cholesterol levels are well known: (1) the formula involves an estimation by calculation; (2) it requires multiple assays and multiple steps, each adding a potential source of error; (3) it is inaccurate as triglyceride levels increase; (4) it requires that patients fast for 12 to 14 hours prior to specimen collection to avoid a triglyceride bias; and (5) it is not standardized.[14,15] The Friedwald formula cannot be applied for people with elevated triglyceride levels (>400 mg/dL or 4.5 mmol/L). In a recent study, LDL cholesterol values calculated by the Friedewald formula were compared with those measured by vertical spin density gradient ultracentrifugation, a laboratory method closely calibrated with beta quantification by one of the CRMLN laboratories.[2] The investigators found that the LDL cholesterol values routinely calculated with the Friedewald formula were progressively lower than directly measured values as triglyceride levels increased. When triglyceride levels were ≥150 mg/dL, the Friedewald formula commonly classified LDL cholesterol as <70 mg/dL despite directly measured levels ≥70 mg/dL.[2]

Because the reference method for LDL cholesterol measurement is not appropriate for the routine clinical laboratory, and because of the drawbacks of the Friedewald formula, as noted before, the expert laboratory panel of the US NCEP called for the commercial development of direct measures of LDL cholesterol.[3]

THE FIRST-GENERATION DIRECT LDL CHOLESTEROL TEST: AN IMMUNOSEPARATION METHOD

The first available direct LDL cholesterol test — the Direct LDL Cholesterol Immunoseparation Reagent (Sekisui Diagnostics) — used affinity-purified goat polyclonal antisera to specific human apolipoproteins, which facilitated the removal of HDL and VLDL in the specimen. After centrifugation, LDL remained in the filtrate solution. The LDL cholesterol concentration was obtained by performing an enzymatic cholesterol assay on the filtrate solution. The advantages of this method were that it offered a direct quantitation of LDL cholesterol from one measurement, it did not require patient fasting, and it provided accurate LDL cholesterol measurements even in the presence of elevated triglycerides. However, the test was not fully automated, and it was cumbersome to perform.

THE SECOND-GENERATION DIRECT LDL CHOLESTEROL TEST: THE FULLY AUTOMATED HOMOGENEOUS METHOD

In 1997, the N-geneous LDL cholesterol test was introduced as the first automated homogeneous LDL cholesterol test in the United States. 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 offered significant cost savings and ease of use for laboratories. The N-geneous LDL test met the NCEP performance goals for LDL cholesterol testing in terms of inaccuracy, imprecision, and total error.

N-geneous LDL Cholesterol Test

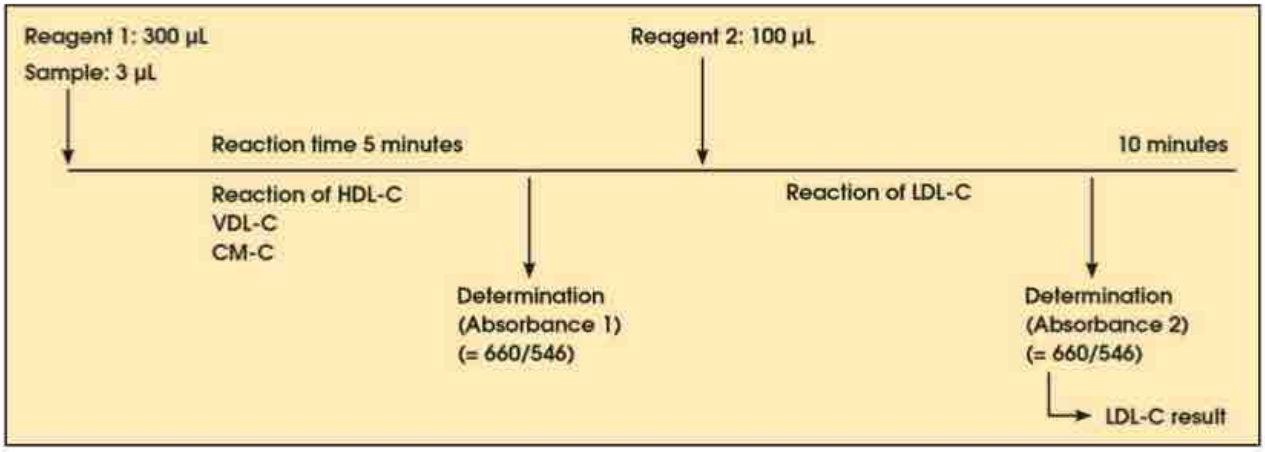
ASSAY PROCEDURE

N-geneous LDL is a liquid two-reagent homogeneous method for the direct measurement of LDL cholesterol. The method is completely automated and applicable to most clinical chemistry analyzers. It does not require any off-line pretreatment, centrifugation, or reagent-reconstitution steps. **Figure 1** provides a general schematic about how the N-geneous LDL test procedure works on an automated analyzer and the length of time it takes.

Figure 1. A schematic of the N-geneous LDL test procedure for an automated analyzer.

ASSAY PRINCIPLE

N-geneous LDL is a two-reagent format that depends on the properties of a unique detergent. The detergent (reagent 1) hydrolizes only the non-LDL lipoprotein particles. The cholesterol that is released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (reagent 2) then solubilizes the remaining LDL particles, and a chromogenic coupler allows for color formation. Cholesterol color development is proportional to the amount of LDL cholesterol present in the sample. **Figure 2** depicts the two-reagent effects of the N-geneous LDL cholesterol test.



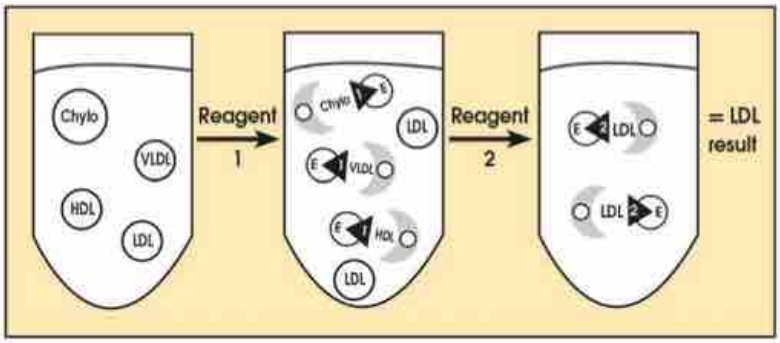


Figure 2. A graphic depiction of the two-reagent format and process of the N-geneous LDL cholesterol test.

- Detergent 1
- Cholesterol esterase
- Cholesterol oxidase
- Detergent 2

LDL test was substantially equivalent to the beta-quantification reference method and to the first-generation Direct LDL immunoseparation test.

To evaluate the effect of high levels of triglycerides on the comparative performance of the N-geneous LDL test versus beta-quantification, 19 patient serum samples with endogenous triglyceride values ranging from 407 mg/dL to 1293 mg/dL were added to the original 54 normotriglyceridemic samples. **Figure 4** depicts the resultant slope, intercept, correlation coefficient, and corresponding scatter plot for all samples. This analysis showed that the N-geneous methods had acceptable correlation with beta-quantification even with samples exceeding 400 mg/dL of triglyceride.

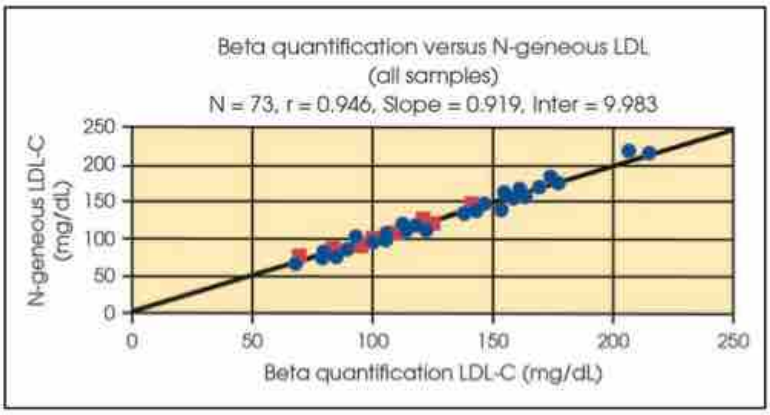


Figure 4. Regression equation and correlation with 19 patient serum samples that have endogenous triglyceride values ranging from 407 mg/dL to 1293 mg/dL for the N-geneous LDL test versus beta-quantification.

● = normal serum; ■ = high triglyceride

PRECISION

Within-run and between-run precision studies were conducted with the N-geneous LDL Cholesterol test using frozen serum pools at three levels of LDL cholesterol: <130 mg/dL (low); 130 mg/dL to 159 mg/dL (mid); and ≥160 mg/dL (high). The within-run results, derived from 20 samples at each of the three LDL cholesterol levels, are shown in **Table 3.** Overall, the N-geneous LDL test had a within-run CV for precision of ≤0.73%. Between-run precision studies were run for 10 days, with the N-geneous LDL test run in duplicate and analyzed twice per day. The between-run precision results, derived from 40 sample results at each of the three LDL cholesterol levels, are also shown in **Table 3.** Overall, the N-geneous LDL test had a between-run CV for precision of ≤2.27%.

Table 3. Within-run and between-run precision studies of N-geneous LDL Cholesterol reagents

Serum pool	<130 mg/dL	130–159 mg/dL	≥160 mg/dL
Within-run precision studies			
n	20	20	20
Mean LDL level mg/dL (sd)	98.1 (0.72)	146.5 (0.96)	209.8 (1.31)
CV (%)	0.73%	0.66%	0.62%
Between-run precision studies			
n	40	40	40
Mean LDL level mg/dL (sd)	98.1 (2.2)	142.7 (2.8)	207.3 (3.6)
CV (%)	2.27%	1.95%	1.73%

CV = coefficient of variation; sd = standard deviation

TOTAL ERROR

Total error is a measure of the overall analytical performance of an assay and combines both accuracy and precision. Total error is equal to the percentage bias + (1.96 X total CV). The percentage bias between the N-geneous LDL test and beta-quantification was calculated using the formula

$$\frac{y - x}{x}$$

Table 4. Total error calculation for the N-geneous LDL test for a sample set including both normal and elevated triglyceride specimens

LDL cholesterol concentration	Bias (%)	Total CV (%)	Total error (%)
100 mg/dL	1.9%	2.4%	6.6%
145 mg/dL	–1.2%	2.4%	5.9%
180 mg/dL	–2.6%	2.4%	7.3%

CV = coefficient of variation

Table 4 depicts the results of the total error analysis for the N-geneous LDL test for a sample set including both normal and elevated triglyceride specimens.

In this analysis, the N-geneous LDL test met accuracy (≤ ±4% bias), precision (≤4% CV), and total error (≤12%) goals at low, medium, and high LDL cholesterol levels in the presence of both normal and elevated triglycerides.

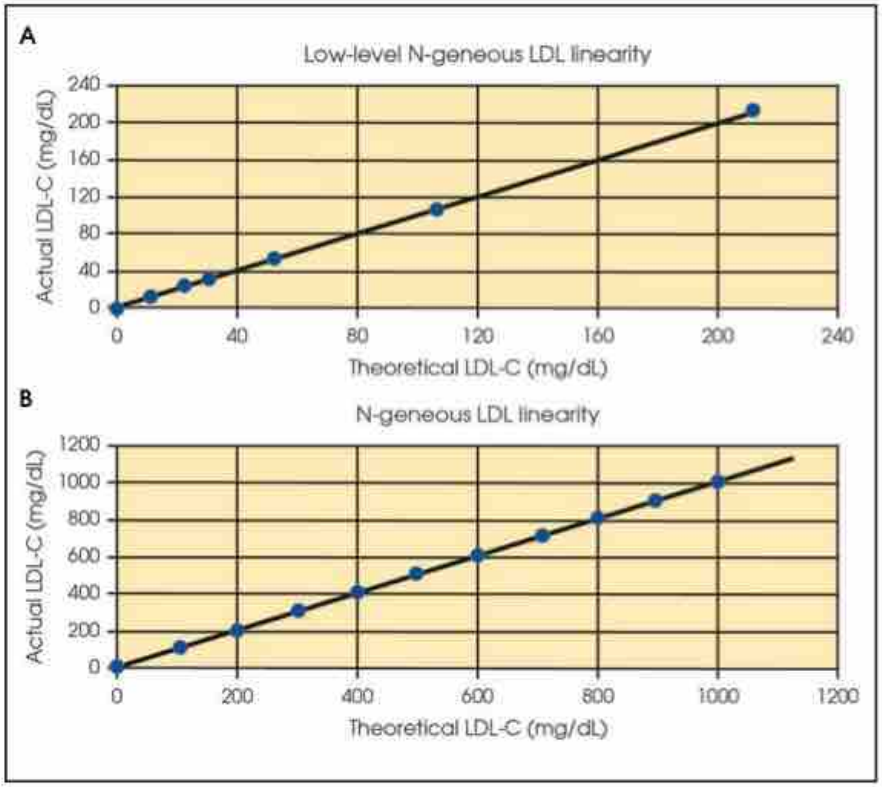


Figure 5. Linearity studies of the N-geneous LDL test using **(A)** natural serum samples and **(B)** delipidated serum pools spiked with human HDL fractions isolated by ultracentrifugation.

LINEARITY

Linearity studies were conducted using natural serum samples and delipidated serum pools spiked with human LDL fractions isolated by ultracentrifugation. Linearity samples were prepared by serially diluting either natural or spiked serum samples with physiological saline. As shown in **Table 5** and in **Figure 5,** results with the N-geneous LDL test were linear between 6.6 mg/dL and 992 mg/dL with a deviation from the linear line ≤3%.

Table 5. Results of linearity studies with the N-geneous LDL test

Theoretical value (mg/dL)	Actual value (mg/dL)	Percent theoretical (%)
Low-level linearity (natural serum samples)		
209.6	209.6	100%
104.8	105.2	100%
52.4	53.5	102%
26.2	26.6	101%
13.1	13.5	103%
6.6	6.5	100%
0.0	-0.1	
N-geneous LDL linearity (spiked delipidated serum)		
992.0	1019.0	103%
892.8	923.5	103%
793.6	818.0	103%
694.4	717.6	103%
595.2	606.7	102%
496.0	508.8	103%
396.8	401.7	101%
297.6	296.3	100%
198.4	195.7	99%
99.2	99.2	100%
0.0	-0.1	

INTERFERENCE

The effect of the interfering substances such as bilirubin, hemoglobin, ascorbic acid, and gamma-globulins on the N-geneous LDL test were evaluated according to the Clinical Laboratory Standards Institute paired-difference method.[16] In these studies, varying levels of potential interferents were added to a specimen pool. **Figure 6** shows the results of the interference studies. Ascorbic acid was found not to interfere with the N-geneous LDL test up to a level of 50 mg/dL, using a criterion of ≤5% versus the control value. For free and conjugated bilirubin, N-geneous LDL test recoveries were within 95% of baseline levels up to 20 mg/dL. Hemoglobin was found not to interfere with the N-geneous LDL test up to a level of 500 mg/dL, using a criterion of ≤5% difference versus the control value. For gamma-globulins at levels up to 5000 mg/dL, N-geneous LDL test recoveries were within 95% of baseline levels.

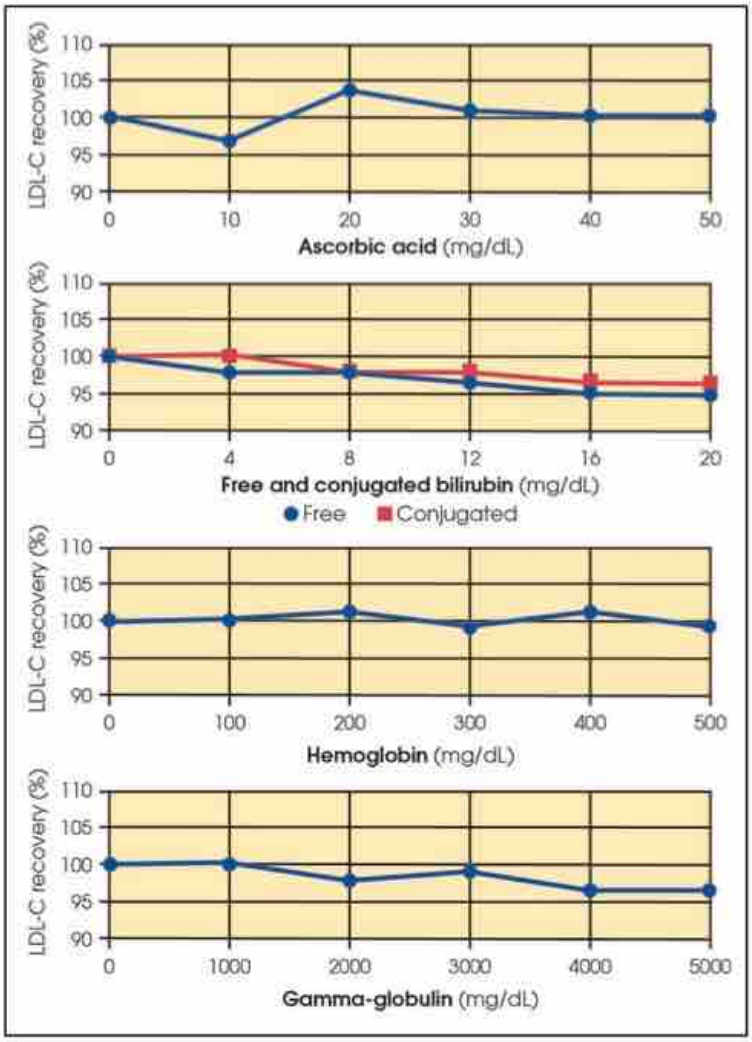


Figure 6. Interference studies for the N-geneous LDL cholesterol test with ascorbic acid, free and conjugated bilirubin, hemoglobin, and gamma-globulins.

SAMPLE TYPE

In conducting sample type studies for the N-geneous LDL test, three samples from each of 10 donors were collected in the following tubes: a serum separator tube, a sodium heparin anticoagulant-coated tube, and an EDTA anticoagulant tube. In this analysis, the LDL cholesterol values from the three different tubes were determined in duplicate, and then the LDL values for the serum-separator tube were compared with those from the sodium heparin tube and the EDTA tube. **Table 6** presents the slopes, intercepts, and correlation coefficients of the comparisons. The mg/dL recovery of LDL cholesterol values generated by N-geneous LDL with sodium heparinized plasma and with EDTA was within 3% of the values generated from serum specimens. All three sample types are recommended for use with the N-geneous LDL test.

Table 6. Results with sample type studies for the N-geneous LDL test

N-geneous LDL test	Serum vs sodium heparin plasma	Serum vs EDTA plasma
n	10	10
Slope	0.97	0.99
Intercept	0.50	–2.47
r	0.99	0.99
Recovery (%)*	98%	97%*

*The EDTA results were corrected by a factor of 1.03X.

FAST-FED

Ten healthy volunteers had serum samples drawn before and approximately 2 hours after consuming a high-fat breakfast. The LDL values of both the fasted and fed serum were determined in duplicate by the test method. **Figure 7** shows the results of the fast-fed study. The N-geneous LDL test was not affected by the dietary status of the patient. For measuring LDL cholesterol values with the N-geneous LDL test, either fasting or fed patient samples can be used.

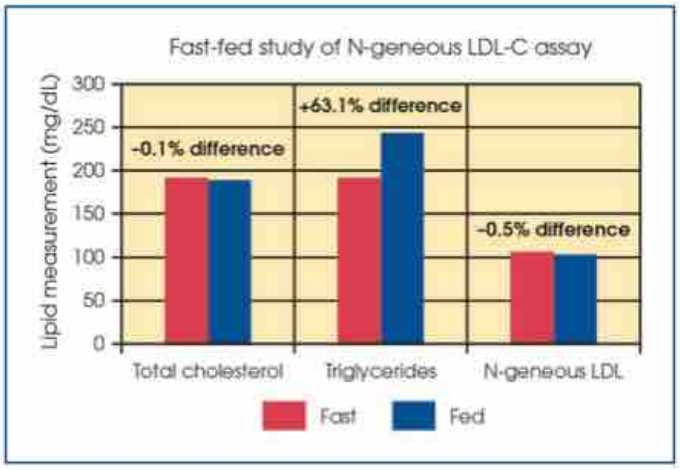


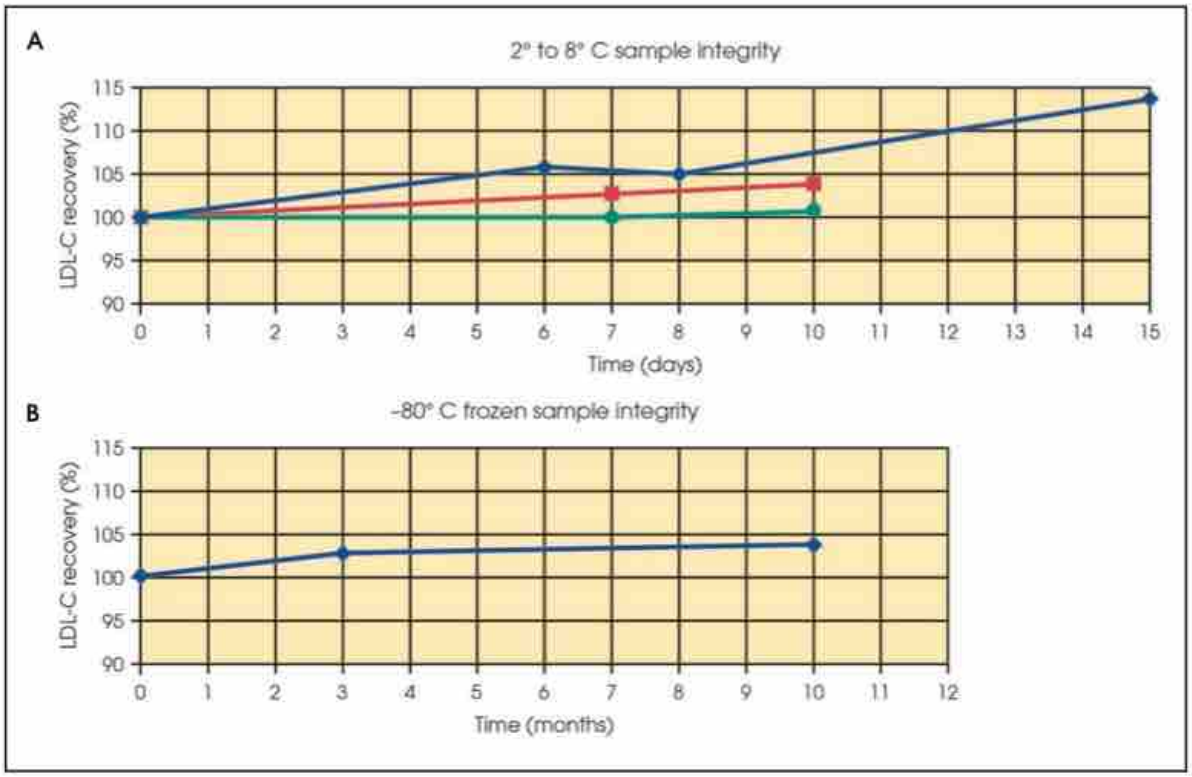
Figure 7. Results of the fast-fed study of the N-geneous LDL cholesterol test in 10 healthy volunteers.

SAMPLE INTEGRITY

The effects of sample aging and of long-term freezing were evaluated using the N-geneous LDL test. LDL cholesterol values of fresh serum and plasma specimens were determined in duplicate. Specimens were subsequently stored at 2° to 8° C and re-tested at intervals of approximately 5 to 6 days over a 2-week period. **Figure 8A** shows the mean percent recovery of the N-geneous LDL test, a result that exceeded the NCEP recommendation of 4 days at 2° to 8° C. For the N-geneous LDL test, it is thus possible to store specimens at 2° to 8° C for up to 5 days.

The effect of long-term freezing of specimens at –80° C was also evaluated. In this evaluation, for which the results are presented in **Figure 8B,** the average recovery on frozen specimens after storage at –80° C for 10 months was 103.1%. Thus, if it is necessary to store specimens for longer than 5 days, they may be stored frozen at –80° C.

Figure 8. Sample integrity studies for the N-geneous LDL cholesterol test **(A)** for specimens stored approximately 5 to 6 days at 2° to 8° C and **(B)** for specimens stored for 10 months at –80° C. ◆ = serum; ■ = heparin; ● = EDTA



WORKING REAGENT STABILITY

Stability for N-geneous LDL reagents 1 and 2 was evaluated open, on board a refrigerated analyzer for periods of 7, 20, and 35 days. All open reagents were paired with freshly reconstituted calibrator and tested against 10 serum specimens. The reported LDL cholesterol values of the open reagents were compared to values obtained from reagents that were stored closed (not open) at 4° C. **Figure 9** depicts the mg/dL recovery of sample LDL cholesterol concentrations using reagents from each of the stability time periods tested. From these results, it can be concluded that N-geneous LDL reagents are stable while open and on-board a refrigerated instrument for at least 4 weeks at 4° C.

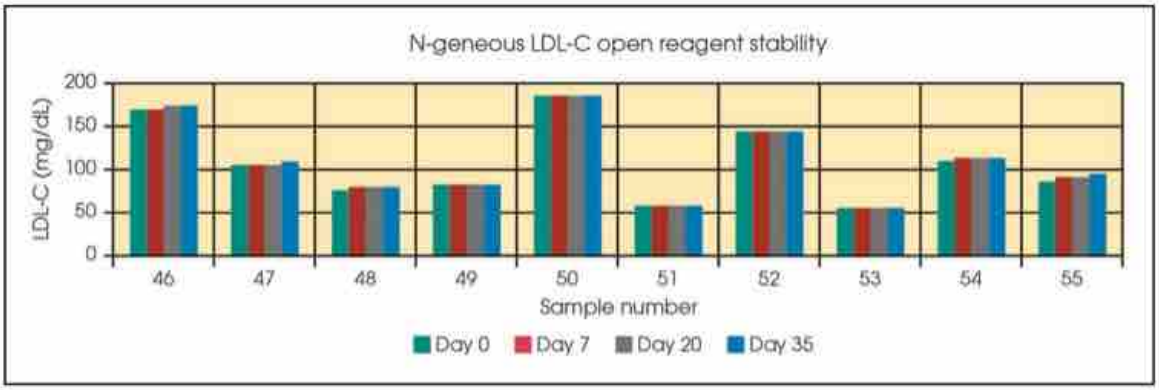


Figure 9. Results of the working reagent stability study of the N-geneous LDL cholesterol test.

REAGENT INTERCHANGEABILITY

Ten samples were tested in duplicate against three lots of N-geneous LDL reagent 1 and three lots of N-geneous LDL reagent 2, paired to form nine unique combinations of reagent 1 and reagent 2. All nine pairs of N-geneous LDL reagents were analyzed in combination, using the same lot of N-geneous LDL calibrator. **Table 7** presents the mean results of samples for each combination of reagent 1 and reagent 2. The minimum result for the combinations was 119.8 mg/dL, and the maximum result was 123.1 mg/dL, an absolute difference of 3.3 mg/dL (2.7%). It can be concluded that different lots of N-geneous LDL reagent 1 and reagent 2 are interchangeable. Any lot of reagent 1 can be used with any lot of reagent 2 in measurement of N-geneous LDL cholesterol.

Table 7. Mean results of the reagent interchangeability studies for the N-geneous LDL test

	Reagent 1 lot A	Reagent 1 lot B	Reagent 1 lot C
Reagent 2 lot A	119.8 mg/dL	122.2 mg/dL	122.8 mg/dL
Reagent 2 lot B	120.9 mg/dL	121.7 mg/dL	122.2 mg/dL
Reagent 2 lot C	120.6 mg/dL	123.1 mg/dL	121.8 mg/dL

CALIBRATOR STABILITY

Individual vials from one lot of N-geneous LDL calibrator were reconstituted and stored at 4° C. At 7 and 14 days after reconstitution, the calibrators were tested with N-geneous LDL reagents and compared to values obtained with a freshly reconstituted calibrator. Reconstituted N-geneous LDL calibrators were also aliquoted and stored frozen at –80° C. At 7, 20, and 35 days after reconstitution, the frozen aliquots were thawed and tested against freshly reconstituted calibrators with the N-geneous LDL reagents. All results were converted into percent recovery of the value generated on day 0 (**Figure 10**).

The reconstituted calibrators yielded a <2% decrease in signal for 4° C (14 days) and an average 2.5% decrease in signal for –80° C (35 days) storage as compared to freshly reconstituted calibrator. Based on these results, the N-geneous LDL calibrator was stable after reconstitution for at least 2 weeks at 4° C. If the calibrator was aliquoted and frozen at –80° C, it was stable for 4 weeks.

Figure 10. Results of the calibrator stability study of the N-geneous LDL cholesterol test.
● = 4° C; ■ = –80° C

Conclusion

The benefits of the N-geneous LDL test are substantial and are summarized in **Table 8.** The test is fully automated and easy to use, with a simple assay principle. The test is accurate and precise, readily meeting the NCEP performance criteria in terms of acceptable imprecision, inaccuracy, and total error. Unlike LDL cholesterol values calculated by the Friedewald formula, results with the N-geneous LDL test are accurate even in the presence of increasing levels of triglycerides and do not require fasting specimens, a great convenience for patients and clinicians. In light of the new ACC/AHA guidelines on treating cholesterol, with their continued emphasis on the LDL cholesterol level as the cornerstone of treatment decisions, the need for real — rather than estimated — measurement of LDL cholesterol is greater than ever.

Table 8. Summary of the features and benefits of the N-geneous LDL test

Features	Benefits
Easy to use	Eliminates all sample pretreatment. Sample size of only 3 µL.
Ready to use	Two lipid stable reagents requiring no reagent preparation. No waste due to reagent preparation errors.
Simple traditional assay principle	Based on selective detergent and standard cholesterol enzymes. Component lot interchangeability (any lot of reagent 1 can be used with any lot of reagent 2).
No patient fasting required	More convenient for patients, physicians, and laboratories. Ensures accurate results regardless of patient fasting status.
Linear between 6.6 mg/dL and 992 mg/dL	Provides confidence in results at both low and high LDL cholesterol levels. Reduces cost of having to dilute specimens with high LDL cholesterol levels.
No interferences	No significant interference from elevated levels of ascorbic acid, bilirubin, hemoglobin, triglycerides, or gamma-globulins
Calibrator traceable to the CDC reference method	Confidence in the accuracy of results and the proper classification of patients.
Precision	Within-run precision <1.0% and between-run precision <3.0%. Exceeds the 1998 NCEP goals for precision.
Accuracy	Excellent correlation to beta-quantification reference method and to the Direct LDL immunoseparation method. Exceeds the 1998 NCEP goals for accuracy.
Performance	Exceeds the 1998 NCEP total error goals for both normal and high triglyceride specimens.

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