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Review

Methods for Measurement of LDL-Cholesterol: A Critical Assessment of Direct Measurement by Homogeneous Assays versus Calculation

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Background: **Because LDL-cholesterol (LDL-C) is a modifiable risk for coronary heart disease, its routine measurement is recommended in the evaluation and management of hypercholesterolemia. We critically examine here the new homogeneous assays for direct determination of LDL-C.**

Approach: **This review relies on published studies and data of the authors using research and routine methods for LDL-C determination. We review experience with methods from their earlier use in lipid research laboratories through the transition to routine clinical testing and the recent development of homogeneous assays. We focus on comparative evaluations and characterizations and the performance of the assays.**

Content: **Homogeneous assays seem to be able to meet current National Cholesterol Education Program (NCEP) requirements for LDL-C testing for precision (CV <4%) and accuracy (bias <4%), when samples collected from nonfasting individuals are used. In addition, all five currently available assays have been certified by the Cholesterol Reference Methods Laboratory Network. The homogeneous methods also appear to better classify individuals into NCEP cutpoints than the Friedewald calculation. However, the limited evaluations to date raise questions about their reliability and specificity, especially in samples with atypical lipoproteins.**

Conclusions: **Available evidence supports recommending the homogeneous assays for LDL-C to supplement the Friedewald calculation in those cases where the calculation is known to be unreliable, e.g., triglycerides >4000 mg/L. Before the homogeneous assays can be confidently recommended to replace the calculation in routine practice, more evaluation is needed.**
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The association between total cholesterol (TC)[4] and risk of developing coronary heart disease (CHD) has been well established by studies such as the Framingham Heart Study. Most of the cholesterol in circulation is carried by LDL, which has been conclusively shown by many prospective studies and randomized clinical trials to be primarily responsible for the association with CHD risk *(1, 2)*. Intervention studies performed in patients with (secondary prevention) *(3, 4)* and without (primary prevention) clinically manifested CHD *(5–7)* clearly demonstrated the efficacy of lipid-lowering therapies even at relatively low LDL-cholesterol (LDL-C) concentrations.

The recently updated NIH-sponsored National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) guidelines, which provide a comprehensive overview of clinical evidence, maintain the focus of diagnosis and treatment efforts on TC and LDL-C, with more attention to primary prevention in persons with symptoms of atherosclerotic disease, diabetes, and multiple risk factors, especially those associated with the metabolic syndrome *(1)*. Therapy is targeted on lowering LDL-C values below a target value, which depends on the

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[4] Nonstandard abbreviations: TC, total cholesterol; CHD, coronary heart disease; NCEP, National Cholesterol Education Program; IDL, intermediate-density lipoprotein; HDL-C, LDL-C, IDL-C, and VLDL-C, HDL-, LDL-, IDL-, and VLDL-cholesterol, respectively; TG, triglyceride; Lp(a), lipoprotein(a); apo, apolipoprotein; RM, Reference Method; LRC, Lipid Research Clinics; BQ, β-quantification; CRMLN, Cholesterol Reference Methods Laboratory Network; HLP, hyperlipoproteinemia; Lp(a)-C, Lp(a)-cholesterol; CAP, College of American Pathologists; POE-POP, polyoxyethylene-polyoxypropylene block polyether; HLB, hydrophile:lipophile balance; SOL, solubilization LDL-C method; Lp-X, lipoprotein-X; PPV, positive predictive value; NPV, negative predictive value; SUR, surfactant LDL-C method; PRO, protecting LDL-C method; CAT, catalase LDL-C method; and CAL, calixarene LDL-C method.

number of other risk factors [low HDL-cholesterol (HDL-C), cigarette smoking, hypertension, family history of CHD, and male gender] present. For patients at highest risk for CHD or with the highest CHD risk equivalents (the latter considered to be diabetes or a 10-year risk for CHD >20%, calculated from the Framingham risk tables), the goal is to achieve LDL-C <1000 mg/L, now considered an optimal value. For patients with two or more risk factors, the goal is to bring LDL-C to <1300 mg/L, and for those with no or one risk factor, the LDL-C goal is <1600 mg/L *(1)*. Patients hospitalized for a major coronary event should have lipid measurements on admission or within 24 h. An NCEP Expert Panel on Blood Cholesterol Levels in Children and Adolescents developed guidelines for detecting, evaluating, and treating children and adolescents with high concentrations of TC and LDL-C *(8)*. Reliable classification of patients necessitates accuracy and standardization of LDL-C measurements.

The most common approach to determining LDL-C in the clinical laboratory is the Friedewald calculation, which estimates LDL-C from measurements of TC, triglycerides (TGs), and HDL-C. Although convenient, the Friedewald calculation suffers from several well-established limitations, which led an Expert Panel convened by the NCEP to recommend development of accurate direct LDL-C methods *(9)*. Earlier direct methods had limitations for general use. Recently, a new generation of homogeneous methods capable of full automation has been introduced that uses specific reagents of various types to selectively expose and directly measure the cholesterol associated with LDL.

This review critically examines the new homogeneous assays within the historical context of the evolution of LDL-C methods. Biochemical and physical characteristics of LDL that facilitate separations are described. Consensus opinions regarding the clinical utility of LDL-C are presented together with guidelines for use in characterizing and treating dyslipidemias. In addition, the characteristics of the various assay systems are summarized in relation to nationally established analytical performance criteria and participation in programs to establish traceability to the accepted targets for accuracy. A timely and important question, whether homogeneous assays can be recommended to replace the Friedewald calculation, is also addressed.

LDL Characteristics

The LDL class comprises a heterogeneous and polydisperse population of particles with sizes between the large TG-enriched VLDL (density <1.006 kg/L) and the dense and small protein-rich HDL (density range, 1.063–1.21 kg/L). Classically, LDL particles are defined in terms of hydrated density as the fraction with density between 1.006 and 1.063 kg/L as obtained by preparative ultracentrifugation. This so-called "broad-cut" LDL fraction is heterogeneous, containing several different lipoproteins: intermediate-density lipoprotein (IDL), with a hydrated density of 1.006–1.019 kg/L and including chylomicrons and VLDL remnants; the main LDL, with a hydrated density of 1.019–1.063 kg/L; and lipoprotein(a) [Lp(a)], an LDL-like particle in which apolipoprotein(a) [apo(a)] is connected by one or more disulfide bonds to apo B-100 *(2)*. On preparative ultracentrifugation, most of the Lp(a) is in the density range of 1.05–1.12 kg/L, thus overlapping with LDL and HDL_2 *(10)*. On an agarose gel at pH 8.6, most LDL particles migrate to the β region (β lipoprotein) and Lp(a) comigrates to the pre-β region together with VLDL, whereas IDL forms a broad band between β and pre-β. In practice, the fractions separated by electrophoresis, chemical precipitation, and chromatography are often simply referred to as LDL, although they do not exactly correspond to those by ultracentrifugation. Thus, LDL particles are defined operationally in terms of the analytical procedure used to isolate them and include a family of similar particles that vary in size and composition.

The heterogeneity of LDL extends beyond IDL and Lp(a) with several different subparticle classes. Proposed nomenclatures for the subclasses are based on density or size, determined by ultracentrifugation or polyacrylamide gel electrophoresis. Small, dense LDL subfractions have been shown to be more atherogenic than the larger, buoyant LDL subfractions *(11, 12)*. A subclass of remnant-like particles, determined after immunoseparation of LDL and nascent VLDL by monoclonal antibodies to apo B-100 and separation of chylomicrons and HDL by monoclonal antibodies to apo A-I, was also shown to be predictive of CHD risk *(13, 14)*.

Of the various lipoproteins, LDL particles have the highest proportion of cholesterol and transport ~70% of plasma TC. The major structural protein is apo B-100, which is rich in positively charged arginine residues. Each LDL, IDL, Lp(a), and VLDL particle contains one molecule of apo B-100. HDL particles do not contain apo B-100. Therefore, apo B-100 determination in VLDL-free serum is a measure of the number of LDL particles. The C apolipoproteins (C-I, C-II, and C-III) are also present in LDL in trace amounts and in IDL, along with apo E, in relatively small amounts.

Cholesterol is the principal lipid component of normal human LDL, comprising >50% of total lipid, including cholesterol esters (~40%) and unesterified cholesterol (~15%). The TG and phospholipid content is ~10% and ~15%, respectively. Because most analytical procedures hydrolyze cholesterol esters, the esterified portion is usually quantified as unesterified cholesterol.

Of particular interest in the context of the homogeneous assays is the fractionation of the subspecies: Lp(a), IDL, and the TG-enriched VLDL and chylomicrons. The concentration of the latter particles is diet dependent and can interfere with some LDL methods, discussed in more detail subsequently.

Recommendations for Analytical Performance

A NCEP expert laboratory panel in 1995 issued recommendations for measurement of LDL-C, emphasizing the importance of accuracy and providing analytical performance goals similar to those for TC, TGs, and HDL-C *(9)*. The panel recommended standardization of the measurements, i.e., achieving traceability of all results to accepted Reference Methods (RMs). The CDC uses an RM, based on the Lipid Research Clinics (LRC) β-quantification procedure (BQ). The CDC BQ method subjects serum to ultracentrifugation at density 1.006 kg/L for at least 18 h at 105 000*g* to float VLDL and any chylomicrons present. A tube-slicing technique removes VLDL and the bottom fraction, which are recovered quantitatively by pipetting and reconstituted to the original volume. HDL-C is obtained according to the HDL RM using heparin-Mn^{2+} to precipitate LDL, including IDL and Lp(a), from the BQ infranate *(15)*. Cholesterol is measured in the fractions by the Abell–Kendall RM *(16)*. LDL-C is calculated by difference as the 1.006 kg/L bottom cholesterol minus HDL-C.

It is important to recognize that the RM has not been formally credentialed or demonstrated to separate the fraction of LDL particles most predictive of CHD risk. The primary rationale for selecting the CDC RM as the accuracy target is to maintain consistency in both research and patient results over time and with the earlier population studies from which medical decision points were taken. For more than a decade, the CDC in collaboration with the National Heart, Lung, and Blood Institute has offered a Lipid Standardization Program (LSP) to provide traceability for cholesterol, HDL-C, and TGs to research laboratories and manufacturers of diagnostic products *(17)*. In 1997, CDC added a program for LDL-C.

The current primary goal for analytical performance in LDL-C is that total error (bias + 1.96CV) be within 12% of the true value. The "total error" term, combining the contributions of imprecision (random error) and inaccuracy or bias (systematic error), represents the maximum tolerable error in measurement of a single specimen to 95% tolerance limits, i.e., in 19 of every 20 measurements. Imprecision, in proportional units, or CV $\leq$4% and bias $\leq$4% from the RM allow a method to meet the current total-error goal.

Commercial control materials, because of changes in their characteristics and matrices during the manufacturing process, may not truly depict performance in patient specimens. Currently, the only approach considered universally reliable for establishing the accuracy of a routine LDL-C assay is a direct comparison study on actual representative patient specimens with the RM. To provide such comparisons, the CDC established a network of experienced laboratories, the Cholesterol Reference Method Laboratory Network (CRMLN), with five laboratories in the US and additional ones throughout the world (described on the AACC website, at www.aacc.org/standards/cdc/cholesterolinfo.stm). All CRMLN laboratories provide traceability to RMs for TC *(17)* and to Designated Comparison Methods for HDL-C. Currently, three CRMLN sites offer traceability to the LDL-C RM. Comparison analyses using fresh human sera follow the EP-9 protocol of the NCCLS *(18)*. Performance within the accepted limits for inaccuracy and imprecision is necessary for certification. Certification is specific to the instrument and application tested. Hence, certification may not be representative of all applications for a particular reagent. Individual clinical laboratories can also arrange to complete comparison studies, which is especially appropriate for a method not certified by the vendor or one that has been modified.

Evolution of Methods for Measurement of LDL-C

ULTRACENTRIFUGATION

Separation of lipoproteins by ultracentrifugation includes both equilibrium and rate methods after adjustment of the specimen density with salts such as NaBr or KBr *(19)* to float or sediment particles based on their differences in hydrated density. As described for the RM above, preparative fractionations can be achieved by subjecting serum or plasma to ultracentrifugation at the native non-protein solute density, ~1.006 kg/L, to float TG-rich VLDL and chylomicrons, which are recovered by tube slicing or by aspirating with a syringe or pipette. The 1.006 kg/L bottom fraction, the infranate containing LDL, HDL, IDL, and Lp(a), can be adjusted to 1.063 kg/L by the addition of a salt such as KBr and resubmitted to ultracentrifugation to float LDL *(20)*, the cholesterol content of which is taken as a measure of LDL. Because the ultracentrifugation steps are tedious and technically demanding, for routine separation of LDL from HDL simpler precipitation procedures were substituted. The common research procedure for LDL-C, combining ultracentrifugation and precipitation, is termed BQ. Recent modifications decrease specimen volumes and separation times, improving convenience *(21)*.

Ultracentrifugation as a separation technique is not only tedious and time-consuming, but the highly labile lipoproteins can be substantially altered by the high salt concentrations and centrifugal forces. Furthermore, a plethora of different types of equipment and tubes are used, making conditions difficult to reproduce from one laboratory to another and consistent separations highly dependent on the skills and care of the technician. Achieving complete and reproducible recovery is difficult, and cross-contamination is common. In addition, the fractions are heterogeneous, containing other functional particles. Nevertheless, ultracentrifugation is still considered the classic comparison method and is the basis for the accepted RM.

ELECTROPHORESIS

Fredrickson and Lees *(22)* introduced a phenotyping system to classify hyperlipoproteinemias (HLPs) based on lipoprotein separation patterns obtained on paper electro-

phoresis, a scheme that was revolutionary at the time but now is primarily of historical interest. Later, more convenient cellulose acetate *(23)* and polyacrylamide *(24)* electrophoresis systems were described. The first quantitative electrophoretic measurement using agarose gels to separate lipoproteins followed by precipitation with polyanions *(25)* and densitometric scanning *(26)* gave reliable values, especially for LDL-C *(27–29)* with a revised algorithm as demonstrated by comparison to BQ *(30)*. Nevertheless, specimens with unusual lipoproteins can produce errors; e.g., comigration of Lp(a) into the pre-β band *(31)*. Agarose electrophoresis was substantially improved by the introduction of enzymatic cholesterol determination using cholesterol esterase, cholesterol dehydrogenase, and nitroblue tetrazolium chloride dye *(32–34)* (Table 1). Comparisons to a modified LRC BQ procedure revealed

Table 1. Methods for separation and quantification of LDL-C.

Procedure	Disadvantages	Advantages
Ultracentrifugation		
Sequential *(20)*	Tedious; large sample volume; relatively imprecise	Allows determination of lipoprotein composition
LRC method (combined with heparin/Mn^{2+} ppt)[a] *(16)*	Tedious; large sample volume	Basis for CDC RM; well standardized; allows the determination of lipoprotein composition; relatively precise
Electrophoresis		
Qualitative paper *(22)*, cellulose acetate *(23)*, polyacrylamide *(24)*	Not quantitative	Easily interpretable lipoprotein profile; allows classification according to Fredrickson
Quantitative agarose gel electrophoresis *(26)*	Requires fresh specimen; Lp(a)-C not resolved	Atypical lipoproteins can be observed; reliable determination of LDL-C concentration
Agarose gel electrophoresis with subsequent enzymatic staining *(32, 34, 36, 38)*	Only semi-automated; requires experience; somewhat technique dependent	Traceable to CDC RM; relatively precise; allows detection of atypical lipoproteins and altered samples; gels can be stored for visual record
Friedewald calculation *(40)*	Well-known limitations: requires fasting; TGs <4000 mg/L; type III HLP excluded; relatively imprecise; accuracy declines with TGs >2000 mg/L; inaccurate with increased TGs, e.g., secondary HLPs	Extensive experience; used in many clinical studies; well-established clinical significance; convenient and inexpensive when TC, TGs, and HDL-C are measured
Homogeneous		
SOL LDL-C method *(100)*	General underestimation of LDL-C, especially at lower concentration range; LDL-C recovery, 97–105%; VLDL not totally excluded; only 52–64% of IDL-C measured; Lp(a)-C not measured?;[b] overestimation of LDL-C in type III HLP; reactive against buoyant and small, dense LDL-C?; apoE-rich HDL-C not totally excluded; affected by freezing?; classification of patients not demonstrated to be better than calculation	CRMLN certified; precise; broad linear range; fully automated; decreased TG interference (negative bias); modest interferences from bilirubin and hemoglobin; Lp(a)-C included?; does not measure Lp-X cholesterol; multicenter evaluations available; postprandial data promising, but not sufficient to recommend
SUR LDL-C method *(111)*	General underestimation of LDL-C; LDL-C recovery, 87%; VLDL not totally excluded; only 31–47% of IDL-C is measured; overestimation of LDL-C in type III HLP; 50% of Lp-X cholesterol also measured; reactive against buoyant and small, dense LDL-C?; apoE-rich HDL-C not totally excluded; freezing?; classification of patients no better than calculation; no multicenter evaluations	CRMLN certified; precise; broad linear range; fully automated; decreased TG interference (positive bias); interferences from bilirubin and hemoglobin significantly reduced; Lp(a)-C included; postprandial data promising, but not sufficient to recommend
PRO LDL-C method *(102, 103)*	LDL-C recovery?; VLDL not totally excluded; IDL-C?; Lp(a)-C?; overestimation of LDL-C in type III HLP; Lp-X cholesterol?; reactivity against buoyant and small, dense LDL-C?; apoE-rich HDL-C?; freezing?; classification of patients no better than calculation; no multicenter evaluations	CRMLN certified; precise; broad linear range; reasonably accurate; fully automated; decreased TG interference (positive bias); interferences from bilirubin and hemoglobin significantly reduced; postprandial data promising, but not sufficient to recommend
CAT LDL-C method *(104)*	LDL-C recovery?; VLDL not totally excluded; only 30% of IDL-C is measured; only 50% of Lp(a)-C is measured; Lp-X cholesterol?; reactivity against buoyant LDL-C decreased; apoE-rich HDL-C?; classification of patients?; LDL-C in type III HLP?; no multicenter evaluations	CRMLN certified; precise; broad linear range; fully automated; decreased TG interference; interferences from bilirubin and hemoglobin significantly reduced; freezing at −80°C acceptable; postprandial data promising, but not sufficient to recommend
CAL LDL-C method *(115)*	No independent evaluations	

[a] ppt, precipitation.
[b] ?, not known.

good agreement for LDL-C (r = 0.98), provided specimens with high Lp(a) concentrations were excluded *(31)*. This approach with the Helena REP system (Helena Laboratories, Beaumont, TX) gave between-day imprecision (CV) of 1.6–12%, depending on lipoprotein concentrations.

A more convenient alternative uses agarose gel modified by addition of a cation such as magnesium, which slows migration of β and pre-β lipoproteins, producing a distinct additional band between pre-β and α lipoproteins, demonstrated to be Lp(a) by immunofixation. Results agree well with an electroimmunoassay for Lp(a) *(35)*. Addition of urea to the gel allowed simultaneous quantification of the β, pre-β, and α fractions as well as Lp(a)-cholesterol [Lp(a)-C] *(36, 37)* with the mobility of Lp(a) independent of apo(a) size polymorphisms. Compared with the modified LRC BQ, the correlation coefficient and slope for the β fraction were 0.962 and 1.006, respectively *(36)*. A recent evaluation observed total errors for LDL-C between 3.4% and 8.9%, within the NCEP goal *(37)*. Eighty-one percent of individuals were classified correctly based on the NCEP medical decision points for LDL-C. Thus, this convenient method can accurately and precisely measure β-fraction cholesterol directly in fresh serum from patients with a wide range of TG values *(37)*.

Recently, an electrophoretic method using cholesterol esterase and cholesterol oxidase with aminoethyl carbazole dye was reported *(38)*. The detection limit was 42 mg/L per band, and the method was linear up to 4000 mg/L cholesterol. Imprecision was reasonable (CV <3.3%). Compared with BQ, the mean bias for β-fraction cholesterol was 2.9%, with a total error of 7.8%. In samples with TG concentrations of 2000–4000 and >4000 mg/L, bias increased to 5.5% and 6.9%, respectively *(38)*. Sixty-eight percent of individuals were classified correctly in relation to NCEP medical decision points for LDL-C. In fact, 95.7% of individuals were classified in the same or the immediately adjacent category *(38)*. Free fatty acids enhanced mobility of lipoproteins, which was remedied by the addition of albumin *(39)*. Storage of specimens for 3 days at 4 °C increased LDL-C by <14 mg/L, but freezing at −20 °C for 12–36 weeks caused larger discrepancies.

Thus, electrophoresis provides not only reliable quantification of the major lipoproteins, but also a visual display useful in observing variant lipoproteins. Electrophoresis is considered the definitive method for detecting the characteristic broad β band in patients with type III HLP. However, compared with the highly automated instruments used for enzymatic and immunochemical assays, electrophoresis is somewhat labor-intensive and technique-sensitive and thus is of more interest to the specialty laboratory than to the high-volume routine laboratory.

FRIEDEWALD LDL-C: ADVANTAGES AND SHORTCOMINGS

In 1972, Friedewald et al. *(40)* published a landmark report describing a formula to estimate LDL-C as an alternative to tedious ultracentrifugation. The calculation was actually proposed for use in epidemiologic studies, but was later rapidly adopted and became the method of choice by routine clinical laboratories, in part for economic reasons; at the time calculated LDL-C was reimbursed *(9)*. This report has become the most frequently cited one in the journal *Clinical Chemistry*, with >3000 citations in two decades *(41)*. Considering its widespread use, a review of the Friedewald calculation is timely, focusing on new observations since the last status report in 1992 *(42)*. Calculation has essentially become the benchmark for routine quantification of LDL-C, one that each new proposed method must surpass to be accepted.

Principle. Because VLDL carries most of the circulating TGs, VLDL-cholesterol (VLDL-C) can be estimated reasonably well from measured total TGs (TG/5 for mg/L units, or TG/2.2 for mmol/L) *(40, 43, 44)*. LDL-C is then calculated as TC − (measured HDL-C + estimated VLDL-C).

Limitations. Shortcomings of the procedure were addressed in the original publication *(40)*: Because chylomicrons contain proportionately less cholesterol relative to TGs than VLDL, their presence leads to overestimation of VLDL-C and underestimation of LDL-C. Because nonfasting specimens often contain traces of chylomicrons, calculation requires a fasting specimen (ideally >12 h). Similarly, as the TG concentration increases, the proportion of cholesterol to TGs in VLDL decreases, giving rise to errors. Therefore, calculation was recommended only for specimens with TGs <4000 mg/L. Type III HLP or dysbetalipoproteinemia, characterized by accumulation of remnant lipoproteins with an increased proportion of cholesterol relative to TGs, also precludes reliable calculation. For example, mean Friedewald LDL-C in 12 type III patients was 2207 mg/L compared with 1207 mg/L by BQ *(45)*.

Variability of Friedewald LDL-C. A major disadvantage in calculating LDL-C is that the variability is a product of the combined variabilities in the three underlying measurements. The NCEP Expert Panel observed in experienced and well-standardized lipid laboratories that total analytical variability in calculated LDL-C averaged ~4.0%, ranging between 2.7% and 6.8% for LDL-C concentrations between 1000 and 2250 mg/L *(46)*. In routine laboratories, variability appeared to be much higher, e.g., eight survey samples of the College of American Pathologists (CAP) Comprehensive Chemistry Survey analyzed in more than 1150 laboratories gave overall CVs averaging ~12% *(46)*. This CV reflects not only imprecision within laboratories, but also method-to-method biases from the many different assays used in TC, TG, and HDL-C determinations.

The Panel, concluding that many routine laboratories would not be able to achieve the requisite analytical performance using the Friedewald calculation, recommended development of more precise direct methods. The TC determinations have the largest effect on variability in the calculation *(47)*. TG values are divided by 5 and HDL-C concentrations are relatively lower, diminishing their impact. Observations from the lipid laboratories as well as from the CAP survey of routine laboratories suggested that CVs for LDL-C were approximately twice those for TC *(46)*. However, increasing TGs contribute progressively more variability to the calculated LDL-C. Adoption of fully automated homogeneous methods for HDL-C is expected to improve imprecision, including the contribution to calculated LDL-C; nevertheless, the conclusions of the NCEP Expert Panel are likely still valid.

Modifications of the original Friedewald formula. Formulas for estimation of VLDL-C are based on the assumption that specimen composition is uniform, i.e., a relatively consistent relationship between VLDL-C and TGs. Since the original publication, many studies including large populations as well as different ethnic groups have attempted to improve the reliability of the Friedewald equation by adjusting the conversion factor *(48–54)*. Some were summarized in the 1992 status report *(42)*. Other reports proposed modifications in the denominator, added a y-intercept *(55–57)*, included apo B-100 measurements *(58)*, or calculated LDL-C over LDL-apo B-100 *(59)*. However, most modifications were appropriate only for a particular segment of the population or were flawed because they failed to provide a comparison with an accurate RM; none of the modifications have been considered to provide sufficient improvement to be recommended for general use, and the original Friedewald calculation has persisted to the present.

Reliability of Friedewald LDL-C with increasing TG concentration. The reliability of the LDL-C estimations decreases considerably with increasing TG concentrations *(60, 61)*. Specimens with TG concentrations <2000 mg/L give best agreement with BQ; 86–92% show deviations of <10% *(60, 61)*. In specimens with TG concentrations of 2000–3000 and 3000–4000 mg/L, concordance decreased to 75% and 61%, respectively. In specimens with TG concentrations of 4000–5000 mg/L, only 41% showed deviations of <10%, and concordance decreased to 20% in specimens with TGs >5000 mg/L *(61)*.

Postprandial effect. With the LRC BQ method, LDL-C decreased significantly, by 8% and 6%, after 3 and 6 h postmeal, respectively. With calculation, the apparent change was much larger, decreasing significantly after 3, 6, and 9 h, by 22–37%, 15%, and 8%, respectively *(62, 63)*. Therefore, reliable calculation requires a fasting specimen; the minimum is 8 h, but up to 12 h is recommended *(62, 63)*.

Classification of patients according to NCEP guidelines using the Friedewald formula. An important indicator of reliability of a method is correct classification of patients by the NCEP medical decision points for LDL-C. Two large studies with ~10 000 participants compared calculated LDL-C with the LRC BQ. With NCEP medical decision points of <1300, 1300–1600, and >1600 mg/L, 86–88% of the participants were classified correctly *(60, 61)*. An additional 5% were classified one medical decision point low, and 6% were classified one medical decision point high. Only 0.4% were misclassified two medical decision points high, and all of those had type III HLP *(60)*. A Finnish study observed that misclassification over two medical decision point levels was also rare, only 2% *(64)*. These studies are encouraging, but both were conducted in experienced specialty lipid laboratories and may not be representative of performance in routine clinical laboratories.

Effects of Lp(a). A modified formula was suggested that involved subtracting Lp(a)-C from calculated LDL-C *(65)*. This practice may be of interest for research, but it is inconsistent with BQ, the RM, and NCEP recommendations for routine quantification *(66)*, which include Lp(a)-C within a broad-cut LDL fraction together with the other atherogenic lipoproteins: Lp(a), IDL, and remnants.

Secondary HLPs. During recent years, several studies *(67–75)* investigated the application of the Friedewald formula in patients with secondary HLPs. These conditions, discussed below individually, are characterized predominantly by increased TGs, which are well known to make the Friedewald calculation less accurate.

Diabetes. The robustness of Friedewald LDL-C in diabetic individuals was investigated in several studies during the last decade *(67–71)*. Rubies-Prat et al. *(68)* showed a concordance of Friedewald LDL-C with BQ with an error <10% in only 49% of diabetic individuals and concluded that the Friedewald equation should not be used for management of lipid abnormalities in patients with type II diabetes mellitus. In contrast, Winocour et al. *(67)* previously considered calculation appropriate in patients with type II diabetes after demonstrating good correlation with BQ (r = 0.98). Hirany et al. *(69)* observed that the Friedewald equation significantly underestimated LDL-C in diabetic individuals by 8% vs BQ, especially in serum samples with TG concentrations >2000 mg/L. However, the correlation coefficient was 0.969, and 68% of Friedewald LDL-C values were within ± 10% of BQ *(69)*. In another study *(70)*, Friedewald LDL-C showed deviations from BQ <5% in 41% and up to 10% in 74% of specimens, respectively. Calculated LDL-C correlated highly with BQ (r = 0.96), with a significant average underestimation of 5.4% with bias independent of hemoglobin A_{1c}. Branchi et al. *(71)* demonstrated a bias of ≥10% between calculated and BQ LDL-C in 34% of diabetic patients and in 26% of samples

from nondiabetic individuals. However, when serum TGs were <2000 mg/L, the inaccuracy of Friedewald LDL-C (absolute bias ≥10%) was found to be similar in the two groups. Thus, the observed discrepancies are likely a result of the increased proportion of diabetic patients with TGs between 2000 and 4000 mg/L rather than to some other specific effect in diabetes *(71)*.

End-stage renal disease. Two studies compared Friedewald LDL-C to BQ in patients with end-stage renal disease *(72, 73)*. In samples collected after a 10-h fast before dialysis, with TGs <4 g/L *(72)* or <2.8 g/L *(73)*, calculated LDL-C was considered reliable. However, ~20% of hemodialysis and continuous ambulatory peritoneal dialysis patients had TG values >4 g/L, making calculation invalid *(72)* and requiring a more robust method, such as BQ.

Hepatic failure. A small study of 47 alcoholic patients with minimal change or cirrhotic liver disease *(74)* observed reasonable agreement between BQ and Friedewald LDL-C provided that grossly hypertriglyceridemic specimens were excluded. Liver disease increases the TG content of LDL and HDL and decreases that in VLDL, making calculated VLDL-C falsely high with underestimation of LDL-C. Thus, for patients with minimal change, calculated LDL-C was biased −16%, and for those with liver cirrhosis, calculated LDL-C was biased −14%, compared with −6% in the control group.

Hormone replacement therapy. The Friedewald equation underestimated LDL-C compared with BQ in hormone-treated women *(75)* by 2–4 mg/L, on average, a discrepancy considered negligible. However, in research studies, to avoid the bias a denominator of 0.17 (mg/L) might be considered for hormone-treated postmenopausal women.

Summary. Previous studies of the Friedewald calculation have determined that at TG concentrations <2000 mg/L, the Friedewald formula can provide a reliable estimate of LDL-C concentration. With TGs of 2000−4000 mg/L, the reliability is considered acceptable in most cases but may be compromised, which is of special concern, in patients with secondary HLP. For individuals with TGs >4000 mg/L, chylomicrons, or type III HLP, the use of the Friedewald equation for LDL-C estimation is not considered valid.

Conclusion. The original Friedewald calculation has generally prevailed for use in routine laboratories despite the well-established limitations, the many suggested modifications, and introduction of various direct methods for LDL-C in spite of the recommendation by the NCEP Expert Panel, the Working Group on Lipoprotein Measurement, that convenient direct methods be developed to achieve the total error limit of ± 12% *(9, 76)*. The Working Group did pragmatically acknowledge continued use of the calculation, despite the known concerns, until well-validated direct methods become available.

Direct Methods for Determination of LDL-C

First-generation methods (using chemical precipitation)

Even before the NCEP recommendation, attempts were made to develop convenient methods for direct separation of LDL. LDL particles were precipitated with reasonable specificity by the addition of certain reagents, such as heparin at pH 5.12 (Merck, Genzyme, and Polymedco) *(57, 77–83)*; polyvinylsulfate (Roche Diagnostics) *(57, 78, 80–82, 84, 85)*; unspecific amphiphatic polymers (BioMerieux) *(57, 78–80, 86–88)*; or dextran sulfate (Progen) *(57, 81, 82, 89)*. After centrifugation to sediment precipitated LDL and cholesterol analysis, LDL-C_{prec} was calculated as the difference between the TC in serum and the supernate (non-LDL). Alternatively, LDL-C_{prec} could be measured directly after dissolving the precipitate.

Nevertheless, these early precipitation procedures for LDL-C did not replace the more convenient Friedewald calculation; evidence was never compelling that precipitation offered appreciable advantages in precision, accuracy, or specificity *(42)*. In addition, free fatty acids (>2 mmol/L) were shown to interfere negatively with precipitation of LDL by dextran sulfate and polyvinylsulfate *(57, 82, 89)*. Serum TGs >4000 mg/L, known to compromise the Friedewald calculation, also interfered with the precipitation methods; indeed, some VLDL and especially VLDL remnants were shown to coprecipitate with all of the commercially available precipitation procedures *(42, 57)*. Recently, the coprecipitation of VLDL remnants by dextran sulfate was exploited in a screening test for type III HLP *(90)*.

Second-generation methods

In 1994, an immunoseparation method, Direct LDL™ (Genzyme Diagnostics and Sigma Diagnostics), became commercially available. The reagent, no longer in distribution, contained polyclonal (goat) antibodies to human apo A-I and apo E bound to polystyrene latex beads and was designed to remove chylomicrons, HDL, VLDL, and IDL particles, allowing direct determination of LDL-C *(91)*.

The separation was reasonably specific for LDL, but traces of VLDL were detected in hypertriglyceridemic samples *(45)*. Only 75% of serum Lp(a)-C was measured with the LDL fraction *(92)* and none of IDL-cholesterol (IDL-C), an obvious difference from BQ *(91, 93)*. Between-run CVs were 2.0–5.2% in different studies *(45, 91, 92, 94, 95)*, with a mean total error of 13.8% (range, 11.8–15.1%) *(95)*. Freezing affected results, although samples could be stored at 4 °C for up to 3 weeks *(95)*. Agreement with BQ was reasonable in specimens from individuals with normocholesterolemia, combined hyperlipidemia, or isolated hypoalphalipoproteinemia *(94)*; in contrast, hypercholesterolemia produced a negative bias (−5.8%). Hypertriglyceridemia gave an average positive bias of 5–12.5% *(45, 83, 94)*, but scatter was fairly evenly distributed high and low with no clear TG-dependent

trend *(91)*, giving reasonable agreement even with TGs >4000 mg/L. Type III HLP gave results equivalent to *(45)* or lower than *(83, 91)* those obtained by BQ. Another potential advantage was the claimed ability to use nonfasting specimens, although postprandial changes might affect reliability of classification.

A simplified LipiDirect Magnetic precipitation procedure (Reference Diagnostics) *(96, 97)* avoided the need for centrifugation by use of heparin-coated magnetic beads at pH 5.1 to remove LDL from serum, with HDL and VLDL remaining in solution. However, separation using heparin was previously shown to have limited specificity *(42, 57)*. The assay showed run-to-run imprecision of 0.7–2.7%. Comparison of 49 specimens to BQ gave the following regression equation: LipiDirect Magnetic LDL-C = 1.054BQ − 50.0 mg/L; r = 0.937. Similar results were observed in comparisons with calculated LDL-C and with immunoseparation *(96)*. Frozen samples from the CAP or the CDC gave mean recoveries of 102.7% and 97%, respectively. By contrast, frozen samples with high VLDL-C gave falsely high LipiDirect Magnetic LDL-C concentrations *(96)*, supporting the limited specificity. When postprandial samples were compared with fasting samples, a small mean difference of −2.3% was observed (1145 vs 1115 mg/L) *(98)*, based on results published only as abstracts *(96–98)*.

In summary, the simplified magnetic precipitation procedure improves specimen handling, but still requires large sample volumes and special equipment and may not be highly specific.

THIRD-GENERATION METHODS (HOMOGENEOUS ASSAYS)

In 1998, following the introduction of homogeneous methods for HDL-C *(99)*, the first of a new generation of homogeneous methods for LDL-C determination was reported from Japan. A major advantage is the capability for full automation in direct determination of LDL-C. Another potential advantage is improved precision from automated pipetting and accurate control of timing and temperature. Homogeneous methods thus have the potential to improve analytical performance and satisfy the recommendation of the NCEP Expert Panel. However, one must consider that the Friedewald LDL-C can be calculated simply from values usually available, obviating the need for additional measurement. The homogeneous methods, therefore, must demonstrate clear advantages in performance and economics to replace calculation in managing patients.

Five homogeneous methods have become commercially available: from Kyowa Medex, Daiichi Pure Chemicals, Wako Pure Chemical Industries, Denka Seiken, and International Reagents Corp. (Fig. 1 and Table 2). The assays contain different detergents and other chemicals, which allow specific blocking or solubilization of lipoprotein classes to achieve specificity for LDL-C. The cholesterol in LDL is measured enzymatically in the same cuvette. All suppliers offer reagent sets, containing two

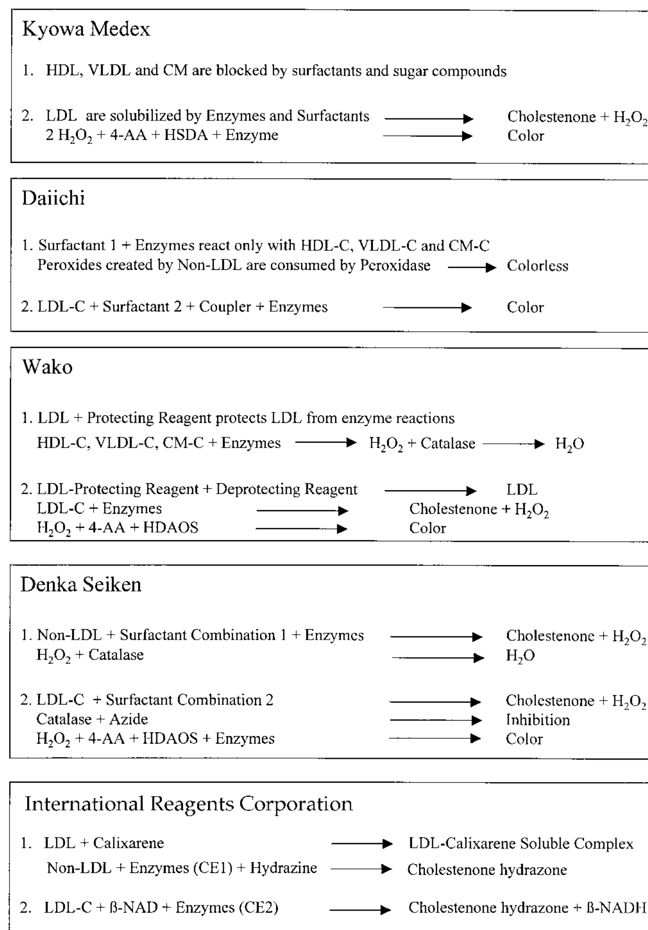

Fig. 1. Principles of homogeneous LDL-C assays.

Schematic reaction mechanisms for each of the five homogeneous methods for LDL-C quantification. *CM*, chylomicrons; *4-AA*, 4-aminoantipyrene; *HSDA*, *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt; *CM-C*, chylomicron-cholesterol; *HDAOS*, *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline.

reagents, that are readily adaptable to most clinical chemistry analyzers.

In 1998, Sugiuchi et al. *(100)* described in detail the mechanisms of an innovative homogeneous method for LDL-C determination and provided the first data about the characteristics of the method offered by Kyowa Medex and distributed by Roche Diagnostics. The reagent set includes two reagents. The first reagent contains $MgCl_2$, dye, buffer (pH 6.75), and α-cyclodextrin sulfate, previously used in their homogeneous HDL-C assay *(101)*, which has a highly concentrated negative charge to mask cholesterol in chylomicrons and VLDL in the presence of magnesium ions *(100, 101)*. Reagent 2 contains the enzymes cholesterol oxidase and cholesterol esterase, peroxidase, dye, buffer (pH 6.75), and a polyoxyethylene-polyoxypropylene block polyether (POE-POP) to block cholesterol, especially in HDL *(100)*. Selectivity to LDL depends on the molecular mass (3850 Da was demonstrated to be optimum) of the POP block in the POE-POP molecule and the hydrophobicity index (90%) *(100)*. A

Table 2. Performance characteristics of homogeneous LDL-C assays.

Method	No. of citations	CV, %	Dynamic range, mg/L	Recovery, %			Accuracy[a]	
				LDL	VLDL	IDL	Bias, %	Bias, mg/L
SOL	8	0.7–3.1	2–4100	97–105	16	52–64	0.8–11.2	−60 to −80
SUR	9	<3.1	4–10 000	87	19	31–47	3.9–5.1	−48 to −80
PRO	1	≤1.2	10–3000	?[b]	?	?	0.4	−15
CAT	2	<1.8	70–5500	95	10	31	?	?
CAL	1	≤0.6	?–4000	?	?	?	?	?

[a] Bias from RM or other equivalent BQ methods. Details are given in the text.
[b] ?, not known.

4-μL serum sample is mixed with 300 μL of reagent 1 and incubated at 37 °C for 5 min before 100 μL of reagent 2 is added. The mixture is incubated for another 5 min, and the chromophore is measured spectrophotometrically at 600 nm (primary) and 700 nm (secondary).

The homogeneous LDL-C assay, from Daiichi Pure Chemicals Company, is distributed by Genzyme Diagnostics. A 3-μL serum sample is incubated with 300 μL of reagent 1 for 5 min at 37 °C. Reagent 1 contains ascorbic acid, oxidase, 4-aminoantipyrene, peroxidase, cholesterol oxidase, cholesterol esterase, buffer (pH 6.3), and a detergent, which solubilizes all non-LDL lipoproteins. The cholesterol reacts with cholesterol esterase and cholesterol oxidase, generating hydrogen peroxide, which is consumed by a peroxidase in the presence of 4-aminoantipyrene with no color generation. Reagent 2 (100 μL) is then added, which contains *N*,*N*-bis-(4-sulfobutyl)-*m*-toluidine disodium salt, buffer (pH 6.3), and a specific detergent, which specifically releases cholesterol from LDL particles. An enzymatic reaction similar to that described above occurs except that the hydrogen peroxide reacts with *N*,*N′*-bis-(4-sulfobutyl)-*m*-toluidine disodium salt to generate a colored product [measured at 546 (main) and 660 (subsidiary) nm] that is proportional to LDL-C.

The homogeneous LDL-C assay from Wako Chemicals, which is distributed by Sigma Diagnostics, contains two ready-to-use reagents. Reagent 1 consists of Good's buffer [pH 6.8; *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt], cholesterol esterase, cholesterol oxidase, catalase, polyanions, and amphoteric surfactants, which selectively protect LDL from enzyme reaction. The non-LDL cholesterol reacts with cholesterol esterase and cholesterol oxidase, producing hydrogen peroxide, which is consumed by catalase. Reagent 2 contains Good's buffer (pH 7.0), 4-aminoantipyrene, peroxidase, sodium azide, and deprotecting reagent. The nonionic surfactants remove the protecting agent from LDL, enabling the specific reaction of cholesterol esterase and cholesterol oxidase with LDL-C. The resulting hydrogen peroxide yields color with Trinder's reagent and 4-aminoantipyrene in the presence of peroxidase. Serum (3 μL) is added to 270 μL of reagent 1 and incubated at 37 °C for 5 min; 90 μL of reagent 2 is then added and incubated for another 5 min. The blue color complex produced has an absorbance peak at ~600 nm and is measured at 600 nm (primary) and 700 nm (secondary) *(102, 103)*.

The fourth homogeneous assay, from Denka Seiken (distributed by Polymedco Inc.), removes non-LDL cholesterol via a selective reaction with cholesterol oxidase and cholesterol esterase, with the resulting peroxide byproduct eliminated by reaction with catalase. Reagent 1 consists of 20 mmol/L $MgCl_2$, 600 U/L cholesterol esterase, 500 U/L cholesterol oxidase, 200 U/L catalase, and 0.6 mmol/L *N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt; 0.24 g/L Emulgen 66 (polyoxyethylene compound; Kao), and 0.6 g/L Emulgen 90 in Good's buffer (100 mmol/L PIPES, pH 7.0). Emulgen 66 and Emulgen 90 are nonionic surfactants, and the hydrophile:lipophile balance (HLB) of the combination was 13.5. Sample (4 μL) is added to 300 μL of reagent 1 and incubated at 37 °C for 5 min, after which 100 μL of reagent 2 is added, containing 2000 U/L peroxidase, 4 mmol/L 4-aminoantipyrene, 1 g/L sodium azide, and 20 mL/L Triton X-100 (HLB = 12.8) in Good's buffer. Sodium azide inhibits catalase in this step. The resulting color is measured at dual wavelengths, 600 nm (primary) and 700 nm (secondary). The reactivity of cholesterol in the different lipoproteins depends largely on the HLB of the detergents. At HLB <12.8, the cholesterol of all lipoproteins is measured. With increasing HLB, reactivity decreases, especially for LDL and to a lesser extent for VLDL and IDL. In contrast, reactivity toward HDL is blocked only at HLB >13.8 *(104)*.

The homogeneous LDL-C assay from International Reagents Corporation, apparently not available in the US, uses 5 μL of serum and 180 μL of reagent 1 with incubation. Calixarene, a detergent, converts LDL to a soluble complex. Cholesterol esters of HDL-C and VLDL-C are preferentially hydrolyzed by a cholesterol esterase (*Chromobacterium*); cholesterol oxidase and hydrazine then convert the accessible cholesterol to cholestenone hydrazone. In a second step, 60 μL of reagent 2 (deoxycholate) is added, breaking up the LDL-calixarene complex and allowing LDL-C to react with the esterase, a dehydrogenase, and β-NAD to yield cholestenone and β-NADH; the latter is measured spectrophotometrically.

Results of published evaluations of these methods will be presented subsequently.

Solubilization LDL-C assay (SOL; Kyowa Medex). The SOL assay has been evaluated in various studies, including comparisons with BQ and one multicenter study.

Imprecision and dynamic range. In all published studies, the imprecision was clearly within the NCEP goal of ≤4% (range, 0.7–3.1%) *(100, 103, 105, 106)*. The detection limit was 2 mg/L, and the method was linear up to at least 4100 mg/L *(100, 103)*.

Specificity. The reactivity of cholesterol decreased with increasing POE-POP concentrations in all lipoprotein fractions in the following order: LDL ≫ VLDL > chylomicrons ≈ HDL. POE-POP affected elution patterns of LDL in gel-filtration chromatography. In the absence of POE-POP, proteins (predominantly apo B-100), cholesterol, and phospholipids eluted together. With POE-POP, two separate peaks were obtained, the first containing mainly apo B-100 and the second containing cholesterol and phospholipids. POE-POP solubilized the LDL lipids into mixed micelles with the peripheral hydrophilic POE blocks in solution and the central hydrophobic POP blocks anchoring the surfactant to the hydrophobic surface of the apolipoproteins, facilitating participation in enzymatic reactions with cholesterol esterase and oxidase *(100)*. POE-POP had little effect on HDL; no separation of proteins and lipids was observed *(100)*. Inclusion of Mg^{2+} and α-cyclodextrin sulfate suppressed the reactivity of cholesterol in TG-rich lipoproteins *(100)*.

IDL was only partially recovered, 52–64% *(107, 108)*, whereas VLDL gave a recovery of 16%, indicating that VLDL is not completely excluded *(108)*. In addition, apo E-rich HDL was not completely excluded, with an average of 17.8% measured by the SOL assay as LDL-C. In contrast, samples with increased Lp(a) did not increase the bias, suggesting that Lp(a)-C is included, consistent with BQ *(100)*. However, a conflicting report concluded that Lp(a)-C was not included with LDL in this assay *(109)*. Addition of lipoprotein-X (Lp-X), using sera from patients with cholestasis, confirmed that the SOL assay did not measure Lp-X cholesterol as LDL-C *(107)*. Type III HLP gave an overestimation of ~30% *(103)*, but the discrepancy was much smaller than that observed with the Friedewald calculation. Nevertheless, SOL cannot be recommended for type III HLP. In general, these studies suggest that the method is not completely specific for LDL particles, indicating the challenges in separation of LDL.

Method comparisons. A multicenter study compared SOL with a BQ procedure performed in three laboratories, all enrolled in the CDC LSP, and two in the Alert Lipid Proficiency survey *(105)*. The Alert program, which provided accurate LDL-C targets by RM on fresh sera to assure reliable assessments of accuracy, supplemented the CDC-National Heart, Lung, and Blood Institute LSP program, which surveys TC, HDL-C, and TGs, but not the BQ LDL-C, and used frozen sera. In the three laboratories, the relationship by linear regression was as follows: SOL = 1.037BQ − 95.8 mg/L; r = 0.968; n = 355. In samples with TGs <4000 mg/L, the slope and y-intercept were nearly unchanged (SOL = 1.035BQ − 111 mg/L; r = 0.957; n = 313), whereas in samples with TGs ≥4000 mg/L, the slope and y-intercept increased (SOL = 1.074BQ − 169 mg/L; r = 0.954; n = 42) *(105)*. Overall the SOL method averaged a 5% negative bias in normo- and hypertriglyceridemic samples. In addition, the SOL method was evaluated in a multilaboratory comparison on 29 samples with target values established by the CDC RM. Aliquots were measured in seven laboratories in the US and Europe with reasonably good agreement, regardless of the type of Hitachi analyzer used; slopes of the regression lines were all >1.00 (1.043–1.110), and y-intercepts were all negative (−41.3 to −81.7 mg/L) *(105)*. Mean biases ranged from −12 to 72 mg/L.

Another evaluation was performed in a laboratory standardized by the CDC LSP and participating in the Alert program *(106)*. For all 134 samples with TG concentrations of 184–12 287 mg/L, the equation of the regression line was: SOL = 1.029BQ − 185.8 mg/L (r = 0.962). In a subset of samples with TGs >4000 mg/L, the slope of the regression line increased (from 1.029 to 1.139), and the negative intercept was more pronounced (−255.4 mg/L). The overall negative bias of −8% indicates either incomplete measurement of the cholesterol content of all the broad-cut LDL fraction or perhaps inaccurate calibration *(106)*.

The SOL was compared with a modified BQ in another laboratory enrolled in the LSP *(103)*. The regression equation was: SOL = 1.052BQ − 168.8 mg/L (r = 0.929; n = 176). A negative systematic error with a mean bias of −95 mg/L was observed with positive correlation between bias and the VLDL-C/TG ratio, which may indicate a source of inaccuracy in the assay *(103)*.

The SOL was also compared with BQ in 86 normolipidemic samples. The equation of the regression line was: SOL = 1.001BQ + 3.87 mg/L (r = 0.989). In this study, the relationships did not seem to vary for normo- or hypertriglyceridemic samples *(100)*. Another study included 100 children with a mean age of 7.7 years (range, 4–12 years). Two blood samples were drawn from each participant within 2 weeks: one after a >9-h fasting period and another in the postprandial state *(110)*. The relationships by linear regression in fasting and nonfasting samples were: SOL = 0.80BQ + 127.7 mg/L (r = 0.891) and SOL = 0.66BQ + 239.4 mg/L (r = 0.747), respectively. The reason for the observed discrepancies with BQ between adults and children is not clear at present, but the results suggest that this method should be used with caution in pediatric populations.

Accuracy. The SOL assay showed consistent negative biases to BQ of −60 to −140 mg/L, independent of LDL-C concentration *(103, 105, 106, 110)*. Esteban-Salan et al. *(103)* observed an average difference of −95 mg/L, with the least negative bias in type IIa HLP (−44.3 mg/L) and the largest negative bias in types IIb and IV, averaging −125 and −129 mg/L, respectively.

Recoveries of added LDL varied between 97% and

105% *(100)*. Differences in systematic errors were observed and decreased with increasing LDL-C concentrations. In different studies, the systematic error of the SOL LDL-C assay ranged from 0.8–6.3% *(105)* to 6.8–11.2% *(106)* and 5.53–10.69% *(103)*. Although the systematic error was often >4%, the SOL assay seemed to meet the NCEP total error target in the range of clinical decisions because of its excellent precision *(103, 105)*. However, in another study, only at high LDL-C concentrations (>1900 mg/L) was the NCEP goal met; the negative systematic error was more pronounced at lower LDL-C concentrations *(106)*.

Classification. For 355 samples from a study in which the SOL homogeneous LDL-C assay met the NCEP analytical performance goals at all concentration ranges, patient classification based on LDL-C medical decision points of 1000 and 1300 mg/L agreed with BQ in 99% and 96% of cases, respectively; between 1300 and 1600 mg/L, classification agreed in 68% of cases; at concentrations >1600 mg/L, classification agreed in 81% of cases, and at concentrations >1900 mg/L, classification agreed in 89% *(105)*. The concordance was dependent on TG concentration and was lowest (85%) at TG concentrations <2000 mg/L. With TG concentrations of 2000–3999, 4000–5999, and ≥6000 mg/L, the concordance increased from 88.9% to >97.5% and 100%, respectively *(105)*. Another approach for judging the reliability of an assay is calculation of the positive (PPV) and negative predictive values (NPV). The PPV of an LDL-C assay at each specific medical decision point was calculated as: [true positive/(true positive + false positive)] × 100, where "true positive" means that the LDL-C results of both compared methods were greater than or equal to the cutoff concentration, and "false positive" means that the test method's LDL-C result was higher than the cutpoint when the BQ procedure LDL-C was below the cutpoint. NPVs were calculated similarly. The PPV of LDL-C decreased with increasing LDL-C concentrations from 100% to 91%, whereas the NPV increased from 80% to 99% *(105)*. In the same study, Friedewald LDL-C was determined in parallel with the SOL assay. The patient classification rates at the same LDL-C medical decision points of <1300, <1600, >1600, and >1900 mg/L were 97%, 97%, 86%, and 91%, respectively. The PPV was practically identical for Friedewald LDL-C and the SOL assay, whereas the NPV was slightly better for Friedewald LDL-C. In summary, the SOL assay did not appear to be superior to Friedewald LDL-C in patient classification.

In another study, because of the systematic underestimation of LDL-C with the SOL assay, especially in those with LDL-C <1300 mg/L, >95% of participants were classified correctly, whereas at LDL-C concentrations of 1300–1600 or >1600 mg/L, <50% were classified correctly *(104)*. In the study by Esteban-Salan et al. *(103)*, increasing TG concentrations gave poorer classification, with concordance decreasing from 98% in samples with TGs <2000 mg/L to just more than 91% in samples with 2000–4000 mg/L, and to 75% in samples with TGs of 4000–10 000 mg/L. The PPVs and NPVs ranged between 90.3% and 99.0% and 74.4% and 96.7%, respectively.

In a pediatric population, the SOL assay classified 91% (fasting) and 89% (nonfasting) of individuals in concordance with BQ at the LDL-C medical decision point of 1100 mg/L and 93% (fasting) and 91% (nonfasting) of individuals at 1300 mg/L, respectively. The Friedewald calculation was in better agreement with BQ when only fasting samples were used, with 96% and 97% of individuals, respectively, classified correctly *(109)*.

TG dependence. The SOL LDL-C assay did not show any relevant interference from high TGs up to 10 000 mg/L. No bias to BQ was observed in samples with high TG or HDL-C concentrations *(100)*. This observation was confirmed in further studies, because the negative bias of the LDL-C SOL to BQ changed only slightly (from 30 to 50 mg/L) at TG concentrations of 1000 mg/L compared with TG concentrations of 10 000 mg/L *(105, 106)*. In samples with TGs >6000 mg/L, the results decreased by >5% *(103)*. In experiments in which isolated VLDL and chylomicrons were added to samples, a negative bias of >10% was found only when TGs exceeded 10 000 mg/L *(105)*.

A comparison between samples collected in the fasting and nonfasting states from 43 individuals revealed a significant decrease in the SOL LDL-C results determined postprandially (1345 vs 1262 mg/L; $P < 0.001$). However, classification of individuals agreed with BQ in 86% (37 of 43) when nonfasting samples were used compared with 83% (36 of 43) in fasting *(105)*. In samples collected from children in the nonfasting state, the negative bias increased slightly compared with samples collected after a 9-h fast (871 vs 894 mg/L) *(109)*. However, the differences did not reach statistical significance *(109)*.

Other interferences. Hemoglobin caused an ~10% positive bias at 6000 mg/L, whereas unconjugated and conjugated bilirubin at 500 and 250 mg/L, respectively, decreased the LDL-C results by <5% *(100, 103, 105)*. Interferences were negligible for bile acids up to 200 μmol/L *(107)*. No adverse effects were seen with ascorbic acid (up to 2.84 mmol/L), Intralipid® (up to 10 g/L), EDTA (up to 0.27 mmol/L), or citrate (up to 0.34 mmol/L) *(100)*. Storage at −20 and −40 °C did not influence the results within a time period of at least 8 weeks *(103)*, with the exception of grossly hypertriglyceridemic samples (>10 000 mg/L), in which a positive bias of 1200–2000 mg/L was observed *(105)*.

Summary. The SOL homogeneous method has been extensively evaluated. Results suggest that the SOL method is reasonably but not completely specific for LDL-C, leading to systematic underestimation, especially at low LDL-C concentrations. The method, even when calibrated appropriately, appears to give a slightly poorer concordance with BQ than the Friedewald calculation in classification of patients. However, in general, the method is more accurate than the Friedewald calculation in type

III patients, in nonfasting individuals, and when TGs are >4000 mg/L.

Surfactant LDL-C assay (SUR; Daiichi). The SUR assay has been evaluated in studies including comparisons with BQ, but not in multicenter studies.

Imprecision and dynamic range. The day-to-day imprecision was ≤3.1% *(111)*. According to the manufacturer, the method is linear up to 10 000 mg/L, and the detection limit is 4 mg/L.

Specificity. A small study (n = 31) with LDL isolated by ultracentrifugation revealed a mean recovery of 87% *(108)*. Although the exact reason for this bias is unknown at present, it seemed to be related to incomplete determination of IDL *(111)*. This hypothesis was confirmed via experiments showing that, on average, only 31–47% of IDL-C was detected after addition *(107, 108)*. Furthermore, the recovery of VLDL was 19%, indicating a lack of specificity *(108)*. In contrast, Lp(a) seemed to be completely included with LDL, which is appropriate. This study was confirmed by the fact that serum with a high Lp(a) concentration did not show an increased discrepancy *(112)*. Other studies revealed that the SUR LDL-C assay measured ~50% of Lp-X cholesterol and 7.6% of apo E-rich HDL-C as LDL-C *(107)*.

Method comparisons. A comparison study by a laboratory participating in the CDC LSP, using a modified BQ procedure, showed a mean bias of −1.2% for six consecutive surveys of the Alert survey program *(111)* and gave the following regression line for 199 serum samples collected from fasting individuals: SUR = 0.91BQ + 34.4 mg/L (r = 0.95), with a mean negative bias of −48.4 mg/L, or −3.8%. In a subgroup of samples with TGs <4000 mg/L, the performance of the assay was worse (SUR = 0.89BQ + 89.8 mg/L; r = 0.94; n = 164) than that seen in samples with TGs ≥4000 mg/L (SUR = 1.02BQ − 29 mg/L; r = 0.96; n = 35). In the two subgroups, the mean biases were −58.4 and −1.6 mg/L, respectively. This study was further confirmed by another evaluation *(106)* in which samples with TGs of 184–12 287 mg/L gave the following regression line: SUR = 0.986BQ − 46.5 mg/L (r = 0.961; n = 134). The SUR assay averaged 5.1% lower values than those obtained by BQ (1289 vs 1358 mg/L; P <0.05). Thus, the SUR assay showed a negative bias of ~80 mg/L, which increased slightly with increasing LDL-C concentrations.

The performance of this assay was also evaluated in 100 children with a mean age of 7.7 years (range, 4–12 years). Blood samples were drawn after a >9-h fasting period *(110)*. The equation of the regression line was: SUR = 0.90BQ + 30.96 mg/L (r = 0.973). The mean bias from BQ was −7.3%.

The performance of the SUR method was compared with that of the Vertical Auto Profile (VAP) method *(113)* in diabetic patients. The equation of the regression line was: SUR = 0.96VAP + 25 mg/L (r = 0.959; n = 52); mean bias was −1.8%. The small negative bias seen was independent of hemoglobin A_{1c} values up to 10.3% *(114)*. Therefore, this homogeneous assay did not seem to be compromised in diabetic patients.

Accuracy. Overall, the SUR method seems to underestimate LDL-C, with the bias being independent of LDL-C concentration *(106, 110, 111)* and ranging from 3.9% to 5.1% at the different NCEP medical decision points. Nevertheless, total analytical error was within the NCEP goals of <12% at all LDL-C medical decision points *(111)* because the excellent precision compensates for the bias *(106)*.

Classification. At LDL-C concentrations <1300 mg/L, 95% of individuals were classified correctly compared with only 60% at LDL-C concentrations of 1300–1600 and >1600 mg/L *(106)*. The classification rate was better in samples with TGs >2000 mg/L (84–87%) than with TGs <2000 mg/L (71%). The PPV ranged from 85% to 100% *(106)* or from 75% to 95% *(111)* and decreased in both studies with increasing LDL-C concentrations. In contrast, the NPV increased with increasing LDL-C concentrations, from 77% to 98% *(106)* and from 84% to 99% *(111)*. When compared with the performance of the Friedewald vs the BQ method in patient classification, the PPV for the SUR assay was equal to or 2% better than the performance of the Friedewald calculation *(106, 111)*. At 1000 mg/L, the NPV was 9% better in one study *(106)*, whereas it was 9% worse in the other *(111)*, but the two methods were within 1–5% at the other LDL-C medical decision points. In a pediatric population, the SUR homogeneous LDL-C assay classified 93% (fasting) and 90% (nonfasting) of individuals correctly at the diagnostic LDL-C decision point of 1100 mg/L; at 1300 mg/L, 98% (fasting) and 97% (nonfasting) of individuals were classified correctly. With the Friedewald formula, 96% and 97% of individuals were classified correctly *(110)*.

Effects of increased TGs. The SUR assay seems to be affected by TGs >4000 mg/L. In samples with TGs >4000 mg/L, the slope of the regression line decreased from 0.986 to 0.925, whereas the negative intercept changed to positive (from −46.5 to 89 mg/L). With increasing TG concentrations, the negative bias of −93 mg/L changed to a positive bias of 120 mg/L *(106)*. Thus, the assay significantly overestimated LDL-C, compared with a modified BQ procedure, when TGs increased. This TG dependence was clearly shown by Horiuchi et al. *(112)*, who observed an overestimation of >10% in 21% of samples with TG concentrations of 1000–1999 mg/L, in 36% with TG concentrations of 2000–2999 mg/L, and in 100% with TG concentrations of 3000–3999 mg/L. The discrepant specimens on lipoprotein electrophoresis revealed a significant TG-enriched lipoprotein fraction between pre-β and β lipoproteins, consistent with the presence of remnants of VLDL and chylomicrons *(112)*. The TG content and density of such lipoproteins vary; some may float in BQ and not be included in the LDL fraction. However, their cholesterol content could be measured by the homogeneous assay as LDL-C, causing the observed discrepancy

(112). In 36 individuals, the mean LDL-C concentration determined postprandially by the SUR was not significantly different from the mean fasting value (1122 vs 1113 mg/L). However, two individuals would be misclassified because of lower results in the postprandial state *(111)*. In children, although the LDL-C measured in nonfasting samples showed a slight but not significant negative bias compared with LDL-C measured in fasting samples (886 vs 870 mg/L) *(110)*, the slope of the regression lines decreased from 0.90 to 0.74 and the *y*-intercept increased from 31 to 159 mg/L. In contrast, addition of sera with isolated TG-rich lipoproteins up to 10 780 mg/L TGs did not significantly change the LDL-C results *(107, 111)*. These studies support the conclusion that some cholesterol of TG-enriched lipoproteins is measured as LDL-C by the SUR assay. TG-enriched lipoproteins are very heterogeneous, and it is not unexpected that some of the chylomicrons, large VLDL, small VLDL, and remnants might react differently with the detergents used in this assay, giving discrepant results.

Other interferences. Hemoglobin up to 10 g/L increased the LDL-C results only slightly *(107, 111)*, whereas bilirubin up to 258 mg/L tended to decrease the results, on average, by <5% *(111)*. Only small and insignificant effects for conjugated and unconjugated bilirubin up to 400 mg/L and bile acids up to 200 μmol/L were observed *(107)*. Ascorbic acid up to 150 mg/L did not alter the results significantly. Storage at 4, −20, or −80 °C up to 4 weeks did not significantly alter results *(95)*.

Summary. The SUR LDL-C assay appears to be somewhat less specific, especially for TG-enriched lipoproteins. Results were especially poor in a pediatric population, at least in the postprandial state *(110)*. However, in overall concordance of patient classification, the SUR assay was approximately equivalent to the Friedewald calculation because of the relatively low systematic bias in samples with low TG concentrations.

Protecting reagent LDL-C assay (PRO; Wako). The PRO assay has been evaluated in one published comparison with BQ, but not in multicenter studies.

Imprecision and dynamic range. Total CVs were 1.2% at LDL-C concentrations between 1034 and 2196 mg/L, and total error ranged from 2.6% to 5.6% *(103)*. The PRO method was linear up to 3000 mg/L and had a detection limit of 10 mg/L.

Method comparison. The PRO was compared with a modified BQ in a laboratory participating in the CDC LSP *(103)*. The equation of the regression line was: PRO = 0.915BQ + 104.8 mg/L (r = 0.969; n = 145). The mean difference was −14.6 mg/L, with a mean bias of −0.4%. The bias was most pronounced in type IIa HLP (−79.3 mg/L), but was only −4 mg/L in normolipidemic individuals *(103)*. A positive correlation between bias and VLDL-C/TG ratio was observed, which may indicate a source of variability in the assay. Furthermore, LDL-C of patients with type III HLP was overestimated by ~30%, indicating that the method is not appropriate for this patient population.

Classification. The TG concentration appears to affect the classification of individuals into NCEP medical decision points; in samples with TG concentrations <2000, 2000–3999, and 4000–10 000 mg/L, 99%, 91%, and 86%, respectively, of individuals were correctly classified. The PPVs and NPVs were 92–100% and 92.5–100%, respectively *(103)*.

Effects of increased TG concentration. In samples with TG concentrations >3000 mg/L, results tended to increase by >5% *(103)*.

Other interferences. Bilirubin up to 234 mg/L and hemoglobin up to 5.3 g/L did not significantly interfere (<5%) with this method *(103)*. Storage at −20 and −40 °C for 8 weeks did not alter the results significantly *(103)*.

Summary. The limited evaluation suggests that the PRO assay seems to be relatively specific for LDL-C, with reservations regarding linearity and TG interference. The classification concordance vs the Friedewald calculation showed a small advantage of the PRO LDL-C assay. However, in samples with increased TG concentrations, the concordance worsened.

Catalase LDL-C assay (CAT; Denka Seiken). The CAT assay has been evaluated in studies including comparisons with the BQ procedure, but not in multicenter evaluations.

Imprecision and dynamic range. The between-assay CV was ≤1.76% over a wide range of LDL-C concentrations *(104)*. The dynamic range of the assay is 70 to at least 5500 mg/L according to the evaluation data of a distributor.

Specificity. LDL prepared by ultracentrifugation was measured by the CAT assay and revealed a mean recovery for LDL of 95% in 31 samples *(108)*. Direct measurements of isolated IDL revealed a recovery of 31%. Recovery of VLDL was 10%, indicating that the assay does not exclude VLDL totally *(108)*. Studies using fast-performance liquid chromatography supported the conclusion that primarily LDL and, to a small extent, VLDL were detected by this homogeneous assay, whereas HDL did not react *(104)*. In addition, Lp(a)-C was measured to the extent of ~50% *(104)*. In a study using isolated LDL subclasses, LDL-I, which corresponds to IDL, showed reduced activity compared with whole serum, whereas LDL-II and LDL-III were comparable to serum values *(104)*.

Method comparison. In 36 samples with TG concentrations <2000 mg/L, comparison with a BQ procedure revealed the following regression line: CAT = 1.01BQ + 38.3 mg/L (r = 0.96). In samples with TGs >4000 mg/L, the equation of the regression line was: CAT = 0.91BQ − 153.5 mg/L (r = 0.86; n = 25) *(104)*.

Effects of increased TG concentration. In hypertriglyceridemic samples, the slope of the regression line decreased, whereas the *y*-intercept increased, indicating reduced specificity. LDL-C measured in samples collected

in the postprandial state compared with samples collected in the fasting state showed an average negative bias of 4.9% *(104)*.

Other interferences. No interference was observed with bilirubin (300 mg/L), hemoglobin (5000 mg/L), ascorbic acid (500 mg/L), or fatty acids (5% soy bean oil) *(104)*. Freezing at −80 °C for 1 week did not change results significantly.

Summary. The very limited evaluation suggests that the CAT assay seems to be relatively specific for LDL-C but that some VLDL-C is measured as LDL-C, indicating interference with high TGs. Studies comparing concordance in classification of patients have not been reported.

Calixarene LDL-C assay (CAL; International Reagents Corporation). There are apparently no evaluations of the CAL assay published in English with the exception of one abstract *(115)* from the manufacturer.

Imprecision and dynamic range. The assay demonstrated a between-assay CV of ≤0.6% at 1525 mg/L and was linear up to 4000 mg/L *(115)*.

Method comparison. A comparison with BQ in 47 fresh sera revealed the following regression line: CAL = 1.02BQ − 28.0 mg/L (*r* = 0.989). In samples with TGs >4000 mg/L (4120–16 420 mg/L), the regression was: CAL = 0.99BQ − 20.9 mg/L (*r* = 0.988; n = 10) *(115)*.

Other interferences. No interference was observed with bilirubin, hemoglobin, and ascorbic acid.

Summary. The very limited evaluation suggests that the CAL assay is promising, but clearly independent studies are needed to better characterize its performance.

Overview of Analytical Performance of Homogeneous Assays

IMPRECISION

Evaluation studies of the homogeneous assays clearly confirm the expected improvements in precision over the earlier methods, especially the Friedewald calculation. Evaluations typically observed between-run imprecision of <3% and total imprecision within the NCEP analytical goal of ≤4% *(100, 103–105, 111)* and far better than the imprecision observed for the Friedewald calculation: ∼4% in expert lipid laboratories and ∼12% in routine laboratories, the latter estimated from CAP surveys *(46)*.

ACCURACY

All of the homogeneous assays have current certifications from the CDC CRMLN (www.aacc.org/standards/cdc/cholesterolinfo.stm). Nevertheless, the demonstrated accuracy may not be applicable to every version of the methods in distribution; lot-to-lot differences, unique calibrations by distributors, different calibrations from country to country, and reformulations of reagents *(99)* might affect accuracy in individual laboratories. Making valid judgments about the accuracy of homogeneous methods based on evaluations in the literature is also challenging. Not all comparisons are made to a BQ method, and even those that are often use variations of BQ protocols that may give different results. In addition, few or no evaluations, especially those published in peer-reviewed publications, are available for some of the reagents. Another complicating factor is the heterogeneous nature of LDL particles, which can behave differently in the various assay systems.

SPECIFICITY AND EFFECT OF INTERFERENCES

Another important aspect of accuracy is the specificity of the homogeneous assays for the LDL-C fraction, which affects accuracy for individual specimens. The available data demonstrate that the homogeneous assays interact unequally with the different components of the broad-cut LDL: LDL subclasses, IDL, Lp(a), and Lp-X *(100, 103, 104, 107, 108, 111, 112)*. Therefore, results for different individuals may vary depending on the reagent, an important consideration in deciding whether to adopt one of the methods, especially for those laboratories supporting lipid research and clinics where a high proportion of unusual specimens from patients may be encountered. The homogeneous LDL-C assays have not yet been rigorously examined with unusual specimens. The reported evaluations were often performed with relatively normal specimens and may not be representative of those from patients with lipid disorders or other conditions, such as liver or kidney disease, with atypical lipoproteins.

Results of conventional interference studies have generally been encouraging. However, specific separation of chylomicrons and VLDL from LDL, IDL, and Lp(a) is much more difficult to accomplish than the separation of apo B-100-containing lipoproteins from HDL. Therefore, it is not surprising that increased TGs often contribute to an increase in apparent LDL-C *(104, 106, 112)*. On the other hand, the SOL assay, which uses sulfated α-cyclodextrin to shield VLDL-C from the LDL-C reaction, usually underestimated LDL-C *(100, 103, 105)*.

A major potential advantage of the homogeneous methods over calculation is the ability to measure LDL-C in specimens collected in a nonfasting state, which is convenient in managing patients. Results, judged by mean differences, are promising. However, patient classification was poorer in the postprandial state *(104, 105, 110)*. Note that all methods comparisons to date have used fasting specimens. Lipoprotein composition is well known to change after a meal; even with BQ changes are often observed. However, changes in vivo are small, and the convenience of nonfasting specimens may offset minor effects on accuracy. Nevertheless, until more comprehensive studies validate the use of nonfasting specimens, laboratories should be cautious about using other than fasting samples. The usual concentrations of bilirubin and hemoglobin do not seem to interfere with these assays.

Considerations in Adopting a Homogeneous Method

The availability of the homogeneous methods for LDL-C, which are capable of full automation and well suited for workflow patterns in the modern clinical laboratory, raises questions about their appropriate use. Should direct measurement replace calculation of LDL-C? Could LDL-C be measured directly in place of TC in monitoring therapy? In which subsets of patients would direct LDL-C be appropriate? Are the direct methods appropriate for research studies? In spite of the technical disadvantages of the Friedewald method—the need for fasting, the relatively high imprecision from the cumulative variations of the three underlying measurements, and the recognized limitations in certain patients—calculation is firmly entrenched in routine practice and will likely be displaced only if the homogeneous methods can demonstrate clear advantages in performance, overall cost-effectiveness, or other financial advantages.

Analytical performance is a key issue. All five homogeneous assays reported to date have current CRMLN certifications, indicating that at least one instrument application has demonstrated acceptable agreement with the RM on relatively normal specimens. Three have been shown to give results comparable to the Friedewald calculation and appear to meet, at least in one study, NCEP performance criteria. Other methods will require additional validation before definitive conclusions about performance can be made. Thus, some of the homogeneous assays could be considered to have demonstrated performance approximately equivalent to calculation in managing the majority of patients. There are, however, important economic considerations to be addressed.

Calculated LDL-C can be obtained without additional cost, provided the underlying values—TC, HDL-C, and TG—are measured, and with adoption of homogeneous methods for HDL-C, all three values are available simultaneously and by fully automated methods. Considering the cost to determine LDL-C alone, reagent costs seem to be approximately equivalent: the homogeneous LDL-C reagents typically cost ~$3.00, which is similar to the total for measurement of TC, TGs, and HDL-C ($0.75 each for TC and TG, and $1.50 for HDL-C). Measuring direct LDL-C in addition to the current panel would double reagent costs ($6.00), and omitting TC saves relatively little ($5.25). The incremental labor costs to include the homogeneous LDL-C assay with the current panel of three assays (TC, TGs, and HDL-C) are insignificant when a modern automated analyzer is used. However, throughput will be decreased with four or even three assays per sample compared with one direct assay, a contribution to overall cost that is more difficult to quantify. Until reagent costs for the direct assays decrease, which might occur with increasing use, a cost advantage of the direct assay for LDL-C over the calculation is not obvious.

In considering whether the panel (TC, TGs, and HDL-C) could be replaced by a single direct LDL-C assay, one must recognize that lipid and lipoprotein analyses are performed primarily in patients for two reasons: (*a*) to screen for hyperlipidemia and (*b*) to monitor treatment of patients with hyperlipidemia. In the first case, determination of LDL-C alone would not detect the risk associated with decreased HDL-C and increased TG concentrations. Although the latter could be detected by observation of turbidity in the specimen, most likely the HDL-C and, in most cases, the TGs would need to be measured in the initial workup. In monitoring therapy, there will be patients, e.g., those with hypercholesterolemia, who could be monitored with only a LDL-C determination. Nevertheless, considering that the common lipid-lowering agents not only reduce LDL-C but also affect TGs and HDL-C, the physician will, in many cases, want all of the values. In summary, a direct measurement only could be adequate for managing therapy in some, but certainly not all, patients, and considering the lack of cost advantage, one must consider whether the occasional direct measurement might complicate practice guidelines and physician decisions about test ordering. The Adult Treatment Panel III recommendation to include TC, HDL-C, and TGs as well as LDL-C in screening all adults *(1)* does not favor replacing calculation with a direct LDL-C measurement.

A thorough assessment of cost-effectiveness would also require consideration not only of the actual measurement costs, but also of the less tangible costs of measurement errors leading to inappropriate treatment decisions. In addition to the direct laboratory costs, there are other indirect costs associated with patient care that are much more challenging to quantify. For example, some of the new homogeneous assays seem to be less affected by TGs than the calculation. Furthermore, measurement in the postprandial state might be possible, despite the known changes that occur in lipoprotein composition after feeding, which would facilitate screening and managing of patients, reduce the need for repeated blood collections, and save time for clinicians, phlebotomists, and patients. An objective assessment of such costs might support the direct measurement, but it would certainly require extensive additional studies, including consideration of outcomes.

An overriding financial consideration in test-ordering practices is reimbursement programs in various countries. A proposed change in reimbursement policy in the US, scheduled for implementation during the year 2002, which would reimburse fewer lipid panels but more related individual tests, may favor adoption of the homogeneous assays for LDL-C irrespective of test performance and other economic considerations.

Trends in Use of Major Conventional and Homogeneous Methods

The homogeneous assays have been available for a relatively short time, and only limited data are available on their use. In the year 2000, proficiency-testing programs in the US began including the homogeneous assays for LDL-C. In addition to the CAP survey, which is the

largest, other smaller programs, such as those of the New York State Department of Health, American Proficiency Institute, and American Association of Bioanalysts, also included data on the homogeneous LDL-C assays. According to the CAP survey, <6% of participating laboratories, primarily in the US, use the homogeneous methods, and <2% use the LipiDirect Magnetic Precipitation procedure. The remaining laboratories (92.7%) use the Friedewald calculation. Cumulative data extracted from the above-mentioned proficiency surveys revealed that of the four available homogeneous methods, the SUR assay from Daiichi was the most widely used (42%), followed by the CAT assay from Denka Seiken (30%), the PRO assay from Wako (18%), and the SOL assay from Kyowa Medex (10%).

Need for Additional Studies

The homogeneous assays are relatively new, and many important performance issues have not been fully evaluated. Laboratories, especially those performing research investigations, long-term clinical trials, and supporting lipid treatment clinics will be cautious about adopting the homogeneous methods until more extensive validations have been completed. The homogeneous methods should be evaluated under representative analytical conditions by comparison with accurate standardized BQ methods, using not only normal specimens but also a broad cross-section of unusual specimens, those at the extremes of the major lipoprotein class distributions and from individuals with conditions likely to alter lipoprotein characteristics (children, elderly, diabetic patients, and patients with liver or kidney disease). Essential in determining the appropriate use in patient characterization and management are studies comparing directly, in representative populations, the analytical performance and concordance in classification of patients by the homogeneous and calculation methods in relation to accurate BQ. The homogeneous methods should also be compared with BQ in a broad cross-section of patients before and after treatment to demonstrate that changes appropriately monitor treatment effects. LDL-C measurements in studies of unusual specimens must be performed in sufficient replicates to distinguish random errors from systematic or matrix-related separation differences. Highly discrepant specimens should be fully characterized to identify lipoprotein and matrix factors responsible for the differences, which might guide assay improvements. Reliability of patient classification and monitoring of therapy using nonfasting specimens should be carefully evaluated. The effects of freezing and prolonged storage of specimens should be determined for each of the homogeneous reagents.

Conclusions

The new homogeneous assays for LDL-C, based on highly innovative assay principles, represent remarkable technologic breakthroughs with great potential for improving lipoprotein analysis, although concerns regarding their performance still exist. The current routine method for LDL-C, the Friedewald calculation, only approximates LDL-C and is subject to well-established limitations. The need for direct, accurate, precise, and convenient replacement methods has been recognized for many years. Studies to date suggest that the homogeneous assays are reasonably specific and free from major endogenous interferences, although additional validation will be needed before the methods can be confidently recommended for general use. The homogeneous methods do appear to be significantly less susceptible to interference from increased TGs than the Friedewald calculation. Furthermore, if these assays could be shown to perform reliably in samples collected postprandially, the measurement of LDL-C will certainly become more convenient for the patient. Evidence available to date justifies recommending the methods for use in patient management for specimens with TGs >4000 mg/L, when calculation is precluded. The methods may be appropriate for certain research studies, although specific validation under the conditions of the study is recommended. Although analytically more challenging and complex than the reasonably well-accepted homogeneous HDL-C assays, the direct LDL-C methods have the potential to significantly improve lipoprotein analyses and simplify the management of patients with HLP. The methods certainly deserve further study to establish the most appropriate applications in quantifying LDL-C.

References

1. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
2. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kammel WB. Incidence of coronary heart disease and lipoprotein levels: the Framingham study. JAMA 1986;256:2835–8.
3. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–9.
4. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 1996;335:1001–9.
5. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA 1984;251:351–64.
6. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365–74.
7. Frick MH, Elo O, Haapa K, Heinonen OP, Heinsalmi P, Helo P, et al. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. N Engl J Med 1987;317:1237–45.
8. National Cholesterol Education Program. Highlights of the report of the expert panel on blood cholesterol levels in children and adolescents. Pediatrics 1992;89:495–584.

9. Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurements of low-density lipoprotein cholesterol: executive summary. National Cholesterol Education Program Working Group on Lipoprotein Measurements. Clin Chem 1995;41:1414–20.
10. Fless GM, Rolih CA, Scanu AM. Heterogeneity of human plasma lipoprotein(a). Isolation and characterization of the lipoprotein subspecies and their apoproteins. J Biol Chem 1984;259: 11470–8.
11. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917–21.
12. Campos H, Genest JJ, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, et al. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12:187–95.
13. Leary E, Wang T, Baker D, Cilla D, Zhong J, Warnick G, et al. Evaluation of an immunoseparation method for quantitative measurement of remnant-like particle-cholesterol in serum and plasma. Clin Chem 1998;44:2490–8.
14. McNamara J, Shah P, Nakajima K, Cupples L, Wilson P, Ordovas J, et al. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. Atherosclerosis 2001;154:229–36.
15. Myers GL, Cooper GR, Henderson LO, Hassemer DJ, Kimberly MM. Standardization of lipid and lipoprotein measurement. In: Rifai N, Warnick GR, Domoniczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 2000;717–48.
16. Cooper GR, Smith SJ, Duncan IW, Mather A, Fellows WD, Foley T, et al. Interlaboratory testing of the transferability of a candidate reference method for total cholesterol in serum. Clin Chem 1986;32:921–9.
17. Myers GL, Kimberly MM, Waymack PP, Smith SJ, Cooper GR, Sampson EJ. A reference method laboratory network for cholesterol: a model for standardization and improvement of clinical laboratory measurements. Clin Chem 2000;46:1762–72.
18. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved guideline. Wayne, PA: NCCLS, 1995.
19. Lindgren F, Jensen L, Hatch FT. The isolation and quantitative analysis of serum lipoproteins. New York: Wiley Interscience, 1972:181–274.
20. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human plasma. J Clin Invest 1955;34:1345–53.
21. Cole T, Ferguson C, Gibson D, Nowatzke W. Optimization of β-quantification methods for high-throughput applications. Clin Chem 2001;47:712–21.
22. Fredrickson DS, Lees RS. A system for phenotyping hyperlipoproteinemia. Circulation 1965;31:321–7.
23. Cobb S, Sanders J. Enzymic determination of cholesterol in serum lipoproteins separated by electrophoresis. Clin Chem 1978;24:1116–20.
24. Muniz H. Measurement of plasma lipoproteins by electrophoresis on polyacrylamide gel. Clin Chem 1977;23:1826–33.
25. Seidel D, Wieland H, Ruppert C. Improved techniques for assessment of plasma lipoprotein patterns. I. Precipitation in gels after electrophoreses with polyanionic compounds. Clin Chem 1973; 19:737–9.
26. Neubeck W, Wieland H, Habenicht A, Müller P, Baggio G, Seidel D. Improved assessment of plasma lipoprotein patterns. III. Direct measurement of lipoproteins after gel-electrophoresis. Clin Chem 1977;23:1296–300.
27. Wieland H, Seidel D. Fortschritte in der Analytik des Lipoproteinmusters. Innere Medizin 1978;5:290–300.
28. Hoffmann G, Schleicher E, Weiss L, Hoffmann S. Comparison of two methods for very low density and low density lipoprotein cholesterol determination. J Clin Chem Clin Biochem 1982;20: 457–60.
29. Bartholome M, Wieland H, Seidel D. Quantification of plasma lipoprotein cholesterol: a simple procedure for enzymatic determination of cholesterol in electrophoretically separated lipoproteins. Clin Chim Acta 1980;104:101–5.
30. Warnick G, Nguyen T, Bergelin R, Wahl P, Albers J. Lipoprotein quantification: an electrophoretic method compared with the Lipid Research Clinics method. Clin Chem 1982;28:2116–20.
31. Nauck M, Wieland H. Measurement of lipoprotein cholesterol with the cholesterol dehydrogenase method after electrophoretic lipoprotein separation. A comparison with quantitative lipoprotein electrophoresis and the ultracentrifugation method. Clin Lab 1993;39:757–65.
32. Urata T. Determination of HDL and LDL-cholesterol by cholesterol dehydrogenase [Letter]. Clin Chem 1983;29:1173.
33. Aufenanger J, Haux P, Weber U, Kattermann R. A specific method for the direct determination of lipoprotein cholesterol in electrophoretic patterns. Clin Chim Acta 1988;177:197–207.
34. Aufenanger J, Haux P, Kattermann R. Improved method for enzymic determination of cholesterol in lipoproteins separated by electrophoresis on thin layer agarose gels. J Clin Chem Clin Biochem 1989;27:807–13.
35. Campos E, Fievet P, Caces E, Fruchart J, Fievet C. A screening method for abnormally high lipoprotein(a) concentrations by agarose lipoprotein electrophoresis. Clin Chim Acta 1994;230: 43–50.
36. Nauck M, Winkler K, März W, Wieland H. Quantitative determination of high-, low-, and very-low-density lipoproteins and lipoprotein(a) by agarose gel electrophoresis and enzymatic cholesterol staining. Clin Chem 1995;41:1761–7.
37. Contois J, Gillmor R, Moore R, Contois L, Macer J, Wu A. Quantitative determination of cholesterol in lipoprotein fractions by electrophoresis. Clin Chim Acta 1999;282:1–14.
38. Benlian P, Cansier C, Hennache G, Khallouf O, Bayer P, Duron F, et al. Comparison of a new method for the direct and simultaneous assessment of LDL- and HDL-cholesterol with ultracentrifugation and established methods. Clin Chem 2000;46:493–505.
39. Nauck M, März W, Wieland H. Adding albumin normalizes electrophoretic mobility of lipoproteins in sera with high concentrations of free fatty acids. Clin Chem 1996;42:1283–5.
40. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499–502.
41. Bruns D. Citation classics in clinical chemistry. Clin Chem 1998;44:698–9.
42. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150–60.
43. Masse J. SI units and the Friedewald formula [Letter]. Ann Intern Med 1992;117:876.
44. Samman S, Truswell A. The Friedewald equation for the determination of low-density-lipoprotein cholesterol: a special case [Letter]. Am J Clin Nutr 1993;58:928–9.
45. Jialal I, Hirany S, Devaraj S, Sherwood T. Comparison of an immunoprecipitation method for direct measurement of LDL-cholesterol with β-quantification (ultracentrifugation). Am J Clin Pathol 1995;104:76–81.
46. National Cholesterol Education Program. Recommendations on lipoprotein measurement. Working Group on Lipoprotein Measurement. NIH Publication No. 95-3044. Bethesda, MD: National

Institutes of Health, National Heart, Lung, and Blood Institute, 1995.
47. Schectman G, Sasse E. Variability of lipid measurements: relevance for the clinician. Clin Chem 1993;39:1495–503.
48. Wilson PW, Abbott RD, Garrison RJ, Castelli WP. Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. Clin Chem 1981;27:2008–10.
49. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, et al. Alcohol and blood lipids. The Cooperative Lipoprotein Phenotyping Study. Lancet 1977;2:153–5.
50. Lippi U, Graziani MS, Manzato F, Schinella M. The Friedewald formula: statistical acrobatics [Letter]? Clin Chem 1986;32: 909–11.
51. Wilson PW, Zech LA, Gregg RE, Schaefer EJ, Hoeg JM, Sprecher DL, et al. Estimation of VLDL cholesterol in hyperlipidemia. Clin Chim Acta 1985;151:285–91.
52. Tyroler HA, Hames CG, Krishan I, Heyden S, Cooper G, Cassel JC. Black-white differences in serum lipids and lipoproteins in Evans County. Prev Med 1975;4:541–9.
53. Hata Y, Nakajima K. Application of Friedewald's LDL-cholesterol estimation formula to serum lipids in the Japanese population. Jpn Circ J 1986;50:1191–200.
54. Fujimoto W. Friedewald's LDL-cholesterol estimation formula in a Japanese American population. Jpn Circ J 1988;52:604–6.
55. Niedbala RS, Schray KJ, Foery R, Clement G. Estimation of low-density lipoprotein by the Friedewald formula and by electrophoresis compared [Letter]. Clin Chem 1985;31:1762–3.
56. Rao A, Parker AH, el-Sheroni NA, Babelly MM. Calculation of low-density lipoprotein cholesterol with use of triglyceride/cholesterol ratios in lipoproteins compared with other calculation methods. Clin Chem 1988;34:2532–4.
57. Nauck M, Friedrich I, Wieland H. Measurement of LDL and VLDL cholesterol with precipitation techniques. A comparison with the ultracentrifugation method. Clin Lab 1994;40:167–76.
58. Planella T, Cortes M, Martinez-Bru C, Gonzalez-Sastre F, Ordonez-Llanos J. Calculation of LDL-cholesterol by using apolipoprotein B for classification of nonchylomicronemic dyslipemia. Clin Chem 1997;43:808–15.
59. Hattori Y, Suzuki M, Tsushima M, Yoshida M, Tokunaga Y, Wang Y, et al. Development of approximate formula for LDL-chol, LDL-apo B and LDL-chol/LDL-apoB as indices of hyperapobetalipoproteinemia and small dense LDL. Atherosclerosis 1998;138: 289–99.
60. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. Clin Chem 1990;36:15–9.
61. McNamara JR, Cohn JS, Wilson PW, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem 1990;36:36–42.
62. Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein cholesterol concentrations in the plasma of human subjects as measured in the fed and fasted states. Clin Chem 1988;34:2456–9.
63. Rifai N, Merrill J, Holly R. Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements. Ann Clin Biochem 1990;27:489–93.
64. Marniemi J, Maki J, Maatela J, Jarvisalo J, Impivaara O. Poor applicability of the Friedewald formula in the assessment of serum LDL cholesterol for clinical purposes. Clin Biochem 1995; 28:285–9.
65. Li KM, Wilcken DE, Dudman NP. Effect of serum lipoprotein(a) on estimation of low-density lipoprotein cholesterol by the Friedewald formula. Clin Chem 1994;40:571–3.
66. Warnick GR. Lipoprotein(a) is included in low-density lipoprotein by NCEP definition [Letter]. Clin Chem 1994;40:2115–6.
67. Winocour P, Ishola M, Durrington P. Validation of the Friedewald formula for the measurement of low density lipoprotein cholesterol in insulin-dependent diabetes mellitus. Clin Chim Acta 1989;179:79–83.
68. Rubies-Prat J, Reverter JL, Senti M, Pedro-Botet J, Salinas I, Lucas A, et al. Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. Diabetes Care 1993;16: 1081–6.
69. Hirany S, Li D, Jialal I. A more valid measurement of low-density lipoprotein cholesterol in diabetic patients. Am J Med 1997;102: 48–53.
70. Whiting M, Shephard M, Tallis G. Measurement of plasma LDL cholesterol in patients with diabetes. Diabetes Care 1997;20: 12–4.
71. Branchi A, Rovellini A, Torri A, Sommariva D. Accuracy of calculated serum low-density lipoprotein cholesterol for the assessment of coronary heart disease risk in NIDDM patients. Diabetes Care 1998;21:1397–402.
72. Nauck M, Krämer-Guth A, Bartens W, März W, Wieland H, Wanner C. Is the determination of LDL cholesterol according to Friedewald accurate in CAPD and HD patients? Clin Nephrol 1996;46: 319–25.
73. Johnson R, McNutt P, MacMahon S, Robson R. Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. Clin Chem 1997;43:2183–4.
74. Matas C, Cabre M, La Ville A, Prats E, Joven J, Turner PR, et al. Limitations of the Friedewald formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease. Clin Chem 1994;40:404–6.
75. Legault C, Stefanick M, Miller V, Marcovina S, Schrott H. Effect of hormone replacement therapy on the validity of the Friedewald equation in postmenopausal women: the Postmenopausal Estrogen/Progestins Interventions (PEPI) trial. J Clin Epidemiol 1999; 52:1187–95.
76. Bachorik P. Measurement of triglycerides and lipoproteins [Review]. Fats Life Newsl 1992;6:9–10.
77. Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res 1983;24:904–9.
78. Demacker PN, Hijmans AG, Brenninkmeijer BJ, Jansen AP, van't Laar A. Five methods for determining low-density lipoprotein cholesterol compared. Clin Chem 1984;30:1797–800.
79. Assmann G, Jabs HU, Nolte W, Schriewer H. Precipitation of LDL with sulphopolyanions: a comparison of two methods for LDL cholesterol determination. J Clin Chem Clin Biochem 1984;22: 781–5.
80. Mulder K, van Leeuwen C, Schouten JA, van Gent CM, Snel MT, Lahey J, et al. An evaluation of three commercial methods for the determination of LDL-cholesterol. Clin Chim Acta 1984;143:29–35.
81. Siekmeier R, März W, Gross W. Precipitation of LDL with sulfated polyanions: three methods compared. Clin Chim Acta 1988;177: 221–30.
82. Siekmeier R, März W, Gross W. Insufficient accuracy and specificity of polyanion precipitation methods for quantifying low-density lipoproteins. Clin Chem 1990;36:2109–13.
83. Maitra A, Hirany S, Jialal I. Comparison of two assays for measuring LDL cholesterol. Clin Chem 1997;43:1040–7.
84. Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H. LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. Clin Chim Acta 1984;140:77–83.
85. Kerscher L, Schiefer S, Draeger B, Maier J, Ziegenhorn J.

Precipitation methods for the determination of LDL-cholesterol. Clin Biochem 1985;18:118–25.

86. Mainard F, Madec Y. Cholesterol, phospholipid and apoB composition of LDL: comparison of precipitation and ultracentrifugation methods. Ann Biol Clin 1986;44:618–23.

87. Mainard F, Madec Y. Are 'precipitated LDL' really low density lipoproteins? Clin Chim Acta 1987;162:141–6.

88. Shaikh M, Miller NE. Evaluation of a commercial reagent for precipitating human serum low-density lipoprotein. Clin Chim Acta 1985;152:213–7.

89. Armstrong V, Seidel D. Evaluation of a commercial kit for the determination of LDL cholesterol in serum based on precipitation of LDL with dextran sulfate. Ärztl Lab 1985;31:904–9.

90. Nauck M, Glatt L, März W, Feussner G, Wieland H. A simple precipitation-based method for the screening of type III hyperlipoproteinemia. Clin Chem 1999;45:909–11.

91. Pisani T, Gebski C, Leary E, Warnick G, Ollington J. Accurate direct determination of low-density lipoprotein cholesterol using an immunoseparation reagent and enzymatic cholesterol assay. Arch Pathol Lab Med 1995;119:1127–35.

92. Cobbaert C, Broodman I, Swart G, Hoogerbrugge N. Performance of a direct, immunoseparation based LDL-cholesterol method compared to Friedewald calculation and a polyvinyl sulphate precipitation method. Eur J Clin Chem Clin Biochem 1995;33: 417–24.

93. Vrga L, Contacos C, Li S, Sullivan D. Comparison of methods for measurement of apolipoprotein B and cholesterol in low-density lipoproteins. Clin Chem 1997;43:390–3.

94. McNamara JR, Cole TG, Contois JH, Ferguson CA, Ordovas JM, Schaefer EJ. Immunoseparation method for measuring low-density lipoprotein cholesterol directly from serum evaluated. Clin Chem 1995;41:232–40.

95. Harris N, Neufeld EJ, Newburger JW, Ticho B, Baker A, Ginsburg GS, et al. Analytical performance and clinical utility of a direct LDL-cholesterol assay in a hyperlipidemic pediatric population. Clin Chem 1996;42:1182–8.

96. Lawlor J, Pelczar D, Sane R. Performance characteristics of the Lipidirect magnetic LDL cholesterol reagent [Abstract]. Clin Chem 1997;43:S262.

97. Sane R, Ward J, McLean M, Lintz A. LDL-cholesterol: the application of magnetic on-line separation and analysis of LDL-cholesterol on the Vitros 950 and Paramax 720 chemistry analyzers [Abstract]. Clin Chem 1997;43:S246.

98. Pelczar D, Lawlor J. Evaluation of the Lipidirect magnetic LDL cholesterol in non-fasting adults. Clin Chem 1997;43:S262.

99. Warnick G, Nauck M, Rifai N. Evolution of methods for measurement of high-density lipoprotein cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem 2001;47:1579–96.

100. Sugiuchi H, Irie T, Uji Y, Ueno T, Chaen T, Uekama K, et al. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and α-cyclodextrin sulfate. Clin Chem 1998;44:522–31.

101. Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated α-cyclodextrin. Clin Chem 1995;41:717–23.

102. Miki Y. A homogeneous assay for the selective measurement of LDL-cholesterol in serum. Enzymatic selective protection method. Clin Lab 1999;45:398–401.

103. Esteban-Salan M, Guimon-Bardesi A, de La Viuda-Unzueta J, Azcarate-Ania M, Pascual-Usandizaga P, Amoroto-Del-Rio E. Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients. Clin Chem 2000;46: 1121–31.

104. Okada M, Matsui H, Ito Y, Fujiwara A, Inano K. Low-density lipoprotein cholesterol can be chemically measured: a new superior method. J Lab Clin Med 1998;132:195–201.

105. Nauck M, Graziani M, Bruton D, Cobbaert C, Cole T, Lefevre F, et al. Analytical and clinical performance of a detergent-based homogeneous LDL cholesterol assay: a multicenter evaluation. Clin Chem 2000;46:506–14.

106. Nauck M, Rifai N. Analytical performance and clinical efficacy of three routine procedures for LDL cholesterol measurement compared with the ultracentrifugation-dextran sulfate-Mg(2+) method. Clin Chim Acta 2000;294:77–92.

107. Fei H, Maeda S, Kirii H, Fujigaki S, Maekawa N, Fujii H, et al. Evaluation of two different homogeneous assays for LDL-cholesterol in lipoprotein-X-positive serum. Clin Chem 2000;46: 1351–6.

108. Sakaue T, Hirano T, Yoshino G, Sakai K, Takeuchi H, Adachi M. Reactions of direct LDL-cholesterol assays with pure LDL fraction and IDL: comparison of three homogeneous methods. Clin Chim Acta 2000;295:97–106.

109. Ordóñez-Llanos J, María Wägner A, Bonet-Marqués R, Sánchez-Quesada J, Blanco-Vaca F, González-Sastre F. Which cholesterol are we measuring with the Roche direct, homogeneous LDL-C Plus assay? Clin Chem 2001;47:124–6.

110. Yu H, Markowitz R. De Ferranti S, Neufeld E, Farrow G, Bernstein H, et al. Direct measurement of LDL-C in children: performance of two surfactant-based methods in a general pediatric population. Clin Biochem 2000;33:89–95.

111. Rifai N, Iannotti E, DeAngelis K, Law T. Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg^{2+} method. Clin Chem 1998;44:1242–50.

112. Horiuchi Y, Takanohashi K, Oikawa S, Numabe A, Hishinuma A, Ieiri T. Measurement of serum low density lipoprotein-cholesterol in patients with hypertriglycemia. Electrophoresis 2000; 21:293–6.

113. Chung B, Segrest J, Ray M, Brunzell J, Hokanson J, Krauss R, et al. Single vertical spin density gradient ultracentrifugation. Methods Enzymol 1986;128:181–209.

114. Ragland BD, Konrad RJ, Chaffin C, Robinson CA, Hardy RW. Evaluation of a homogeneous direct LDL-cholesterol assay in diabetic patients: effect of glycemic control. Clin Chem 2000; 46:1848–51.

115. Kakuyama T, Ochiai K, Kishi K, Ohta Y, Uemura Y, Kayamori Y, et al. New homogeneous assays for estimation of serum HDL and LDL-cholesterol levels. Clin Chem 2001;47:A55.