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Evolution of LDL-C Testing and Cholesterol Management Guidelines

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Abstract

Accurate assessment of low-density lipoprotein cholesterol (LDL-C) is central to the prevention and treatment of atherosclerotic cardiovascular disease (ASCVD). Recent clinical guidelines have emphasized lower LDL-C targets and the use of non-fasting lipid testing, increasing the need for more reliable LDL-C estimation methods than the traditional Friedewald calculation. The development of modern Martin and Sampson methods has led to improved accuracy across a wider range of LDL-C and triglyceride levels. The most recent Modified Sampson method further enhances performance, particularly at low LDL-C levels. These modern LDL-C methods have been incorporated into up-to-date cholesterol management guidelines, which now recommend modern LDL-C methods over the Friedewald calculation. This review summarizes the evolution of LDL-C testing method and its integration into clinical practice to support more precise ASCVD management.

Introduction

The decline in atherosclerotic cardiovascular disease (ASCVD) mortality in the United States since 2010 is, in large part, attributed to the implementation of lifestyle and risk factor management, with low-density lipoprotein cholesterol (LDL-C) remaining a key target for guiding therapies [1,2]. Since its introduction in 1972, the Friedewald calculation method for estimating LDL-C from the standard lipid panel[3] has been widely adopted by clinical laboratories despite its limitation in accuracy when triglyceride (TG) is high and/or LDL-C is low [4]. However, the landscape of ASCVD prevention and treatment has shifted considerably in recent years. Updated cholesterol-management guidelines continue to lower recommended LDL-C target goals as clinical trials have shown the benefit of more intensive LDL-C reduction [5-8]. In addition, non-fasting lipid testing, which often yields higher TG levels than fasting measurements, has been recommended as the screening test for initial ASCVD risk assessment [9].

As the newer guidelines have emphasized lower LDL-C goals and favor non-fasting lipids, laboratory testing methodologies have simultaneously evolved. Recently, Sampson and colleagues described a modification of the original Sampson calculation method for estimating LDL-C, which may be particularly well suited in the management of high-risk patients [10]. This review explores the history of lipid testing, highlighting the evolution of laboratory LDL-C methods and clinical guidelines on cholesterol management.

Evolvement of Lipid Testing

The reference method for LDL-C, which is performed by the Centers for Disease Control and Prevention and is used in the standardization of all routine clinical assays, is β-quantification [11]. It is based on a combination of separation of lipoproteins by ultracentrifugation and selective precipitation. Several alternative approaches have been developed, including vertical auto profile (VAP), gradient gel electrophoresis, nuclear magnetic resonance, ion mobility, and gel-permeation high-performance liquid chromatography, but most of these advanced methods are labor-intensive, costly, and not suited to the high-throughput needs of routine clinical laboratories [12]. Consequently, most laboratories have relied on the standard lipid panel and reported LDL-C based on the Friedewald calculation method.

To address the limitations of the Friedewald method in the setting of hypertriglyceridemia, automated homogeneous, or direct LDL-C assays were developed in the 1990s. These assays do not require centrifugation and typically use detergent-based, polymer-based, or immune-selective reagents to exclude non-LDL lipoproteins before the enzymatic cholesterol reaction. After their commercialization, many laboratories adopted the practice of reflexing to a direct LDL-C method when TG exceeds 400 mg/dL. Shortcomings of these direct LDL-C assays include: 1. Lack of analytical standardization due to the use of different proprietary reagents. 2. Poor accuracy in dyslipidemia. 3. Failure to meet the National Cholesterol Education Program (NCEP) accuracy criteria and total error goals, particularly in diseased or dyslipidemia populations [13,20].

Despite these well documented shortcomings of direct LDL-C methods, they remained widely used in the absence of better alternatives.

A major shift occurred in 2013 with the introduction of the Martin LDL-C method [14]. It was developed from the Very Large Database of Lipids (VLDbL), which is a large real-world dataset that includes a wide range of lipid phenotypes. The method was calibrated to VAP, an ultracentrifugation-based method that measures cholesterol in lipoprotein fractions of different densities. The Martin method replaced Friedewald's fixed TG-to-VLDL-C factor with an adjustable value derived from 180 different strata of TG and Non-HDL-C, allowing more precise estimation across varying TG levels. This innovation improved accuracy, particularly in patients with TG 150-400 mg/dL where fixed-factor methods start to fail. The method was later expanded to add an additional 240 cells to the original stratification, resulting in a 420-cell table. This expansion allows the method to estimate LDL-C in patients with higher TG levels between 400 and 799 mg/dL[15]. Despite these improvements, performance can still decline in cases of extreme hypertriglyceridemia or other abnormal lipoprotein composition.

The next major advance was the Sampson equation, which was developed using β-quantification as the reference standard for LDL-C[16]. Unlike earlier methods, it was designed to maintain accuracy across a broader range of TG levels, including values up to 800 mg/dL. The model incorporates nonlinear relationships between TG and very low-density lipoprotein-cholesterol (VLDL-C), improving performance in patients with elevated TG. This equation was recently modified in 2025 for patients with low LDL-C by re-training it with a large number of patients from the FOURIER clinical trial of evolocumab[5], a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor. Compared to the original calculation method, the Modified Sampson method is simpler, because it combines TC and HDL-C into a single Non-HDL-C variable [10] and has no intercept. These changes improve usability while preserving accuracy, especially at low LDL-C levels.

A timeline of these LDL-C method developments is shown in Figure 1. Recent laboratory medicine guidelines have begun to incorporate these advances, highlighting the limitations of both the Friedewald and direct LDL-C methods [17,18]. In addition to the analytical limitations, direct LDL-C methods carry the extra expense of proprietary reagents, which limits their use in point-of-care devices. Therefore, modern LDL-C calculation methods offer a more accurate and cost-effective approach.

Updates on Clinical Guidelines

Regarding lipid testing, the 2018 multi-society guideline issued a Class 2a recommendation stating that, in adults with LDL-C levels below 70 mg/dL, the use of direct LDL-C measurement or a modified LDL-C estimate [14] is reasonable to improve accuracy over the Friedewald formula [19]. The most recent 2026 guideline further advised against the Friedewald equation, establishing a Class I recommendation that LDL-C testing in both adults and children should preferentially use either the extended Martin method [15] or the Sampson method [16]. The guideline criticized the inaccuracy of the Friedewald equation at TG below 400 mg/dL and recommended the Martin or Sampson methods over direct LDL-C assays when TG is ≥400 mg/dL [9].

Another major shift in the 2026 guideline is the explicit numerical LDL-C treatment targets instead of percentage reduction. For secondary prevention, the recommended thresholds are <70 mg/dL or <55 mg/dL depending on risk category. For primary prevention, targets of 100 mg/dL or 70 mg/dL are advised based on baseline LDL-C and predicted risk. Individuals with subclinical atherosclerosis are assigned targets of 100, 70, or 55 mg/dL according

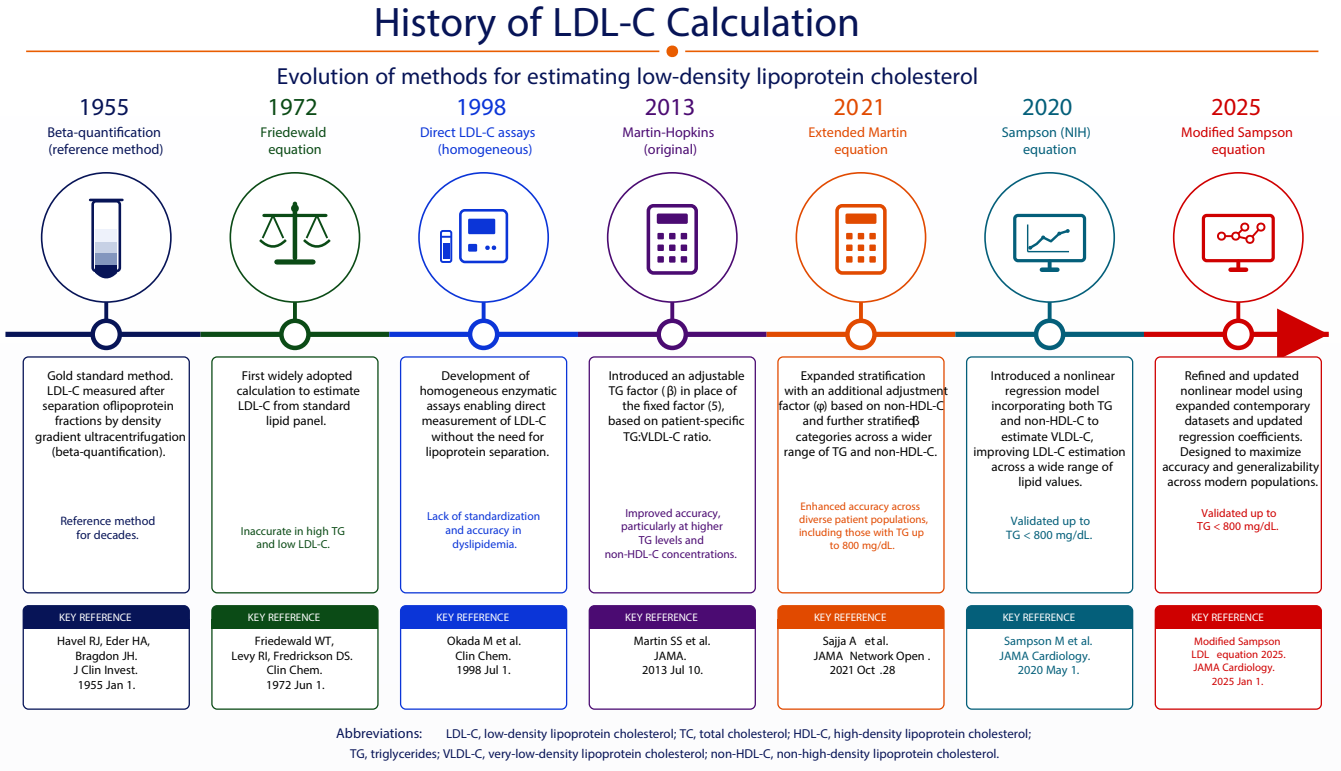

Figure 1. History of LDL-C calculation. Timeline of the evolution of methods for estimating low-density lipoprotein cholesterol, from the beta-quantification reference method (1955) through the Friedewald equation (1972), direct homogeneous LDL-C assays (1998), the original and extended Martin-Hopkins equations (2013, 2021), and the Sampson (2020) and Modified Sampson (2025) equations.

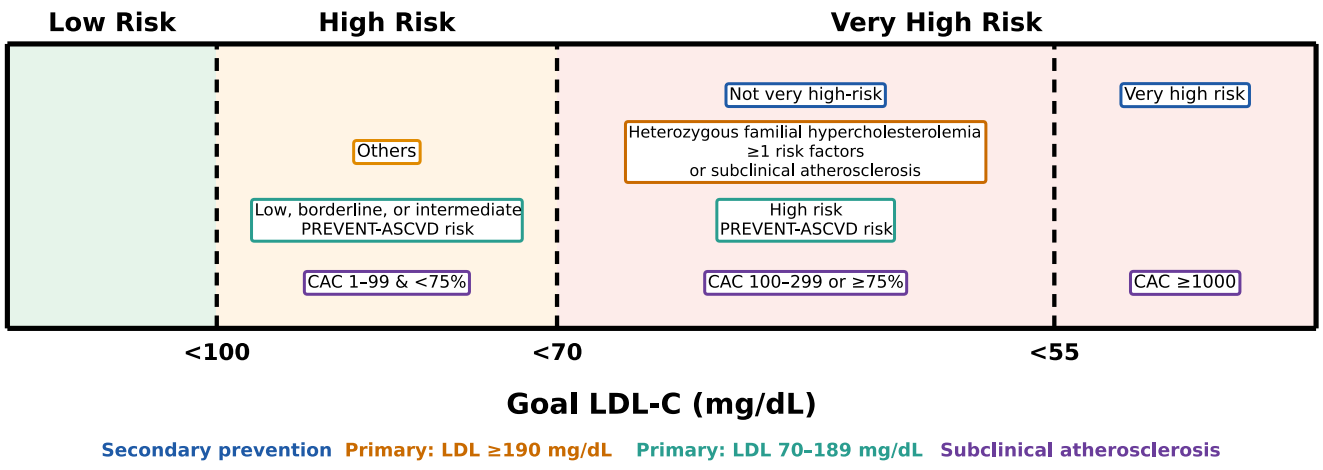

Figure 2. Risk-stratified LDL-C treatment goals under the 2026 multi-society guideline. Goal LDL-C thresholds (<100, <70, and <55 mg/dL) are assigned by risk category (low, high, and very high risk), incorporating PREVENT-ASCVD risk, coronary artery calcium (CAC) score, and clinical criteria for primary and secondary prevention.

to coronary artery calcium (CAC) score (Figure 2). These more aggressive goals have now become feasible with the availability of highly potent PCSK9-inhibitors.

The most recent 2025 update to the Sampson method was not in time for evaluation in the 2026 multi-society guideline. However, Sampson et al 2025 [10] demonstrated that at the key LDL-C thresholds of 55 and 70 mg/dL, the Modified Sampson equation substantially reduced misclassification (mainly under-estimation) compared with the Friedewald method, the Extended Martin method, and the original Sampson equation. Under-estimation of LDL-C places patients at risk for missing considerations of lipid-lowering interventions such as initiation of statin or therapeutic escalation with PCSK9 inhibitors.

A separate 2025 study by Meeusen et al. [20] further proved superior performance of the Modified Sampson

method. Using specimens with high TG (>400 mg/dL) or low LDL-C (<70 mg/dL), the study compared the Modified Sampson method and the Extended Martin method with three commercial direct LDL-C assays against the β-quantification reference method. The Modified Sampson equation resulted in the fewest misclassification at the clinically important thresholds of 55 and 70 mg/dL.

Summary

Advances in lipid testing and the most recent clinical guidelines have made the path forward clear for clinical laboratories: modern calculation methods, such as the Modified Sampson or Extended Martin methods, should be adopted to ensure accurate LDL-C assessment at low concentrations and in non-fasting specimens. Estimating LDL-C by the Friedewald equation should be discontinued to avoid under treatment of patients. Looking ahead, the future of lipid testing in ASCVD prevention will likely rely on fully leveraging not only the standard lipid panel, but also other markers like lipoprotein(a) [Lp(a)] and apolipoprotein B (apoB) [21,22]. Lp(a) is a genetically determined lipoprotein that independently predicts ASCVD risk. ApoB reflects the total number of atherogenic non-HDL particles (including LDL, VLDL, and IDL) as each contains a single apoB molecule, so it provides a direct, highly accurate measure of atherogenic burden and offers superior predictive value over LDL-C. Although cost has historically been cited as a barrier, a recent simulation demonstrated that guiding lipid-lowering therapy using apoB target is highly cost-effective. Broad implementation of this approach could ultimately streamline laboratory assessment of atherogenic lipoproteins and improve clinical management of patients at risk for ASCVD [23].

Declarations and Statements

Conflict of Interest

The authors declare no conflict of interest.

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