ACADEMY ADLM

ADLM Guidance Document on the **Measurement and Reporting of Lipids and Lipoproteins**

AUTHORS

Jing Cao
Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, United States

Leslie Donato
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States

Joe M. El-Khoury
Department of Laboratory Medicine, Yale School of Medicine, New Haven, CT, United States

Anne Goldberg
Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, St. Louis, MO, United States

Jeffrey W. Meeusen *
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States

Alan T. Remaley
Lipoprotein Metabolism Laboratory, Translational Vascular Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, United States

This is a correction to: Jing Cao, Leslie Donato, Joe M El-Khoury, Anne Goldberg, Jeffrey W Meeusen, Alan T Remaley, ADLM Guidance Document on the Measurement and Reporting of Lipids and Lipoproteins, The Journal of Applied Laboratory Medicine, Volume 9, Issue 5, September 2024, Pages 1040–1056, https://doi.org/10.1093/jalm/jfae057

The published article contained the following errors:

- On page 1042, the sentence, “However, it should be noted that desirable has been defined between the 50th and 75th percentiles of healthy populations (Table 1), thus a relatively large proportion of patients will be abnormal” is missing the word “mostly” and should have read: “However, it should be noted that desirable has been mostly defined between the 50th and 75th percentiles of healthy populations (Table 1), thus a relatively large proportion of patients will be abnormal.”
- Also on page 1042, “TC ≤9%” should have read: TC to ≤9%
- In Table 1, Reference value, mg/dL under Pediatrics for Non-HDL cholesterol should have been <120. Reference value, mg/dL under Pediatrics for LDL cholesterol should have been <110.
- On page 1043, the sentence, “Reference intervals for triglycerides were defined in fasting samples based on age-specific percentiles with the 50th percentile defined as normal or acceptable (Table 1)” was missing “in adults” and should have read: “Reference intervals for triglycerides were defined in fasting samples based on age-specific percentiles with the 50th percentile in adults defined as normal or acceptable (Table 1).”
- Also on page 1043, the sentence, “Severe hypertriglyceridemia (>500 mg/dL) raises risk for acute pancreatitis, which can be managed using TG-lowering drugs and or dietary interventions (19)” should have read: “Severe hypertriglyceridemia (>500 mg/dL) raises risk for acute pancreatitis, which can be managed using TG-lowering drugs and/or dietary interventions (19).”
- In Table 4, Recommendation Number 6 should have read as follows, replacing “or” with “and”: “Order sets should allow for both fasting and non-fasting lipid panels, and fasting status should be documented attime of collection and indicated in the result report.”

The article has been corrected.

CITATION Cao J, Donato L, El-Khoury JM, Goldberg A, Meeusen JW, Remaley AT. ADLM guidance document on the measurement and reporting of lipids and lipoproteins. J Appl Lab Med 2024;9:1040-56

TABLE OF CONTENTS

INTRODUCTION

Modern clinical management of atherosclerotic cardiovascular disease (ASCVD) risk depends upon the accurate measurement of blood lipids and lipoproteins. Although great progress has been made in the standardization and harmonization of the methods for the measurement of lipids and lipoproteins, there remains significant heterogeneity in pre-analytical requirements, reference intervals, methods, nomenclature and ordering/reporting work flows. Low-density lipoprotein cholesterol (LDL-C), for example, can be assessed by a wide variety of analytical methods and calculations, each of which has unique limitations or biases. Unfortunately, ambiguous nomenclature in laboratory information systems (LISs) and electronic health records (EHRs) thwart interoperability of LDL-C and other lipid measurements. Some laboratories provide reference intervals as age- and sex-specific normal values, whereas other laboratories report clinical decision thresholds from various clinical guidelines. Finally, there is even less consensus on how to measure and report modern lipoprotein biomarkers like lipoprotein(a) [Lp(a)], apolipoproteinB (apo B), and lipoprotein particle number.

The purpose of this guidance document is to provide an evidence-based reference for use by clinical laboratories to improve standardization of clinical lipid testing work flows. It is our goal that adoption of these recommendations will not only facilitate communication and education among laboratorians but also improve clarity for clinicians and patients

THE LIPID PANEL

What Components Should Be Included in the Lipid Panel?

Measurement of blood lipids is essential in diagnosis and treatment of dyslipidemias and related diseases. Throughout this document, serum and plasma are used interchangably as both are acceptable and considered interchangable sample types for lipid and lipoprotein measurement. Due to the interdependence of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) in patient evaluation, serum lipids are measured as part of a "lipid panel" recognized by the American Medical Association (AMA). In addition to these 3 measures, it is best practice to also calculate and report LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C) (1).

Multiple practice guidelines endorse the use of non-HDL-C (1–4), which is simply cholesterol contained in all lipoproteins except for HDL. Therefore, non-HDL-C is the cholesterol carried by all the atherogenic or potentially atherogenic lipoproteins that contribute to the development and progression of atherosclerotic plaques and can be calculated as the difference between TC and HDL-C (Eq. 1).

$$\text{Non-HDL-C} = \text{TC} - \text{HDL-C} \quad (1)$$

In addition to non-HDL-C, calculated LDL-C should be reported if possible (more details below). Many studies have reported on the potential clinical utility of other calculated parameters based on TC, TG and HDL-C (e.g., ratios and lipoprotein fractions); however, to date, no clinical practice guidelines have endorsed the use of any calculations other than LDL-C and non-HDL-C. In summary, standard lipid panels should report 3 measured values: TC, TG, HDL-C, and 2 calculated parameters: LDL-C and non-HDL-C.

How Should the Lipid Panel Be Named?

Ideally, test names for the lipid panel and its components should indicate the specific analyte being measured, the sample obtained, and method used when appropriate (4). "Total cholesterol" is the preferred term when total serum or plasma

cholesterol is measured. It is important to note that this includes both cholesteryl esters and free or non-esterified cholesterol, which are simultaneously measured by most enzymatic assays for total cholesterol. Cholesterol transported by HDL and LDL are best designated as HDL-cholesterol and LDL-cholesterol or abbreviated as HDL-C and LDL-C, respectively. Finally, because there now exists a wide variety of LDL-C methods in routine use, it is best practice to state either calculated or measured (also known as "direct LDL") in the reporting name. As many estimations for LDL-C now exist (see below), the LDL-C calculation method should also be included in the report comment or laboratory test catalog.

Unlike many other laboratory methods, the ref¬erence values for lipids have been defined based on ASCVD outcomes. The concept of "desirable" lipid values was established by the National Cholesterol Education Program (NCEP) and carried forward by multiple medical societies (1). Reporting a table of lipid thresholds according to ASCVD risk has become common practice for clinical laboratories. However, this may lead to confusion and hinder identification of abnormal results. A single threshold for desirable values corresponding with reduced ASCVD risk is recommended to simplify reporting. However, it should be noted that desirable has been mostly defined between the 50th and 75th percentiles of healthy populations (Table 1), thus a relatively large proportion of patients will be abnormal.

What Are the Expectations for Method Results Agreement and Allowable Error for Lipids?

Based on an expert consensus panel that considered what was analytically achievable and clinically needed, the NCEP established the following total error (bias ± 2SD) goals for the lipid panel tests: TC to ≤9%, LDL-C to ≤12%, and HDL-C to ≤15% (Table 2). There are also further recommendations for the individual bias and imprecision for each of these tests (5, 6).

According to accuracy-based proficiency surveys with fresh frozen plasma, most assays for TC and TG are within the total error limits recommended by the NCEP. Thus, test results from different laboratories that use different methods for TC and TG will usually not differ substantially and can be considered accurate. Direct homogenous assays for HDL-C and LDL-C also yield results within the total error limit set by the NCEP on most samples when lipids are within their typical range (normolipidemic) but can yield discrepant or inaccurate results on dyslipidemic samples (7). Depending on the direct assay, a significant bias (negative or positive) can exist due to lack of selectivity of the method for measuring cholesterol in the

TABLE 1. Recommended reference intervals and commenting thresholds for basic lipid panel parameters.

	ADULTS		PEDIATRICS		
Lipids and lipoproteins	**Reference value, mg/dL[a]**	**Population percentile[b]**	**Reference value, mg/dLa[a]**	**Population percentile[b]**	**Reporting comments**
Total cholesterol	<200	50th	<170	75th	
HDL cholesterol	Female >50 Male >40	50th	>45	25th	<15 mg/dL refer to lipid specialist[c]
Triglycerides	<150	50th	Age 2–9 years < 75 Age 10–18 years < 90	75th	
Non-HDL cholesterol	<130	50th	<120	75th	>220 consider inherited hyperlipidemia[c]
LDL cholesterol	<100	50th	<110	90th	>190 consider familial hypercholesterolemia[c]
Apolipoprotein B	<90	50th	<90	90th	
Lp(a)	<30 mg/dL < 75 nmol/L		NA	NA	

NA, not applicable.
[a]Reference values established by NCEP and carried forward by US multi-society guidelines.
[b]Percentiles based on 2017 National Health and Nutrition Examination Survey (NHANES) data.
[c]HDL 2.5th percentile, non-HDL-C 95th percentile, LDL-C 95th percentile.

TABLE 2. Recommended minimal analytical performance specifications for clinical lipid and lipoprotein methods.			
	Total error, %	**Bias, %**	**CV, %**
Cholesterol	≤9	≤±3	≤3
Triglycerides	≤15	≤±5	≤5
HDL cholesterol	≤12	≤±5	≤4
LDL cholesterol	≤12	≤±4	≤4

lipoprotein fraction of interest. Caution should be used when comparing HDL-C and LDL-C measured by different methods, particularly on sam¬ples with high TG (7). It is important to note that most commercial proficiency testing materials are not based on unmodified human plasma and therefore cannot be assumed to be commutable with clinical samples. These commercial proficiency testing results can only be used to compare performance to a peer group for a particular method and do not provide an adequate assessment of accuracy in terms of bias.

Is Fasting Required for Lipid Assessment?

Prior to the 2018 multi-society US guideline on the management of blood cholesterol (1), it was generally recommended that a fasting sample be obtained for lipid analysis. This was largely driven by the well-established post-prandial increase in TG (8–11). LDL-C calculated by the Friedewald equation significantly underestimates actual LDL-C when TG is elevated (12). Fortunately, as described below, modern equations for estimating LDL-C are less vulnerable to inaccuracy caused by elevated TG.

Recent clinical guidelines have lifted the fasting requirement for initial assessment of ASCVD risk based on multiple lines of evidence. First, large studies have repeatedly demonstrated that TC, HDL-C, and LDL-C are minimally affected by fasting, and that the post-prandial increases in TG are modest for most patients (Table 3) (8–11). Furthermore, longitudinal studies have shown that lipids from a non-fasting blood sample improve ASCVD risk prediction (13, 14). Finally, new LDL-C calculations are less affected by TG (15, 16).

Scenarios remain in which a fasting blood sample is required. It is recommended that adult pa¬tients with hypertriglyceridemia and pediatric patients with elevated non-HDL-C should be re¬tested on a sample collected after fasting at least 8 h (1, 17). Laboratories should work with their practice to build order sets, which allow for lipid panels to be collected both fasting or non-fasting to suit specific patient and provider needs. To prevent confusion in the mixed reporting of fasting and non-fasting results we recommend that the patient fasting status be documented at the time of collection and reported with lipids to aid interpretation (Fig. 1).

How Should Lipid Panel Test Results Be Reported by Clinical Laboratories?

Triglycerides. Chylomicrons and very-low-density lipoproteins (VLDL) are the major carriers of TG. Elevated TG are associated with diabetes, metabolic syndrome, and obesity. Despite these associations, and several large epidemiology studies and Mendelian randomization studies showing associations between TG and ASCVD, a direct and causal relationship between TG and ASCVD is not yet fully established (18). Measurement of TG also helps differentiate certain types of hereditary dyslipidemias like familial chylomicronemia syndrome, familial combined hyperlipidemia, and familial hypertriglyceridemia. Furthermore, TG is also used by different equations for estimating LDL-C.

Reference intervals for triglycerides were defined in fasting samples based on age-specific percentiles with the 50th percentile in adults defined as normal or acceptable (Table 1). Persistently elevated TG ≥ 175 mg/dL are considered a risk-enhancing factor to be considered when managing ASCVD prevention (1). Severe hypertriglyceridemia (>500 mg/dL) raises risk for acute pancreatitis, which can be managed using TG-lowering drugs and/or dietary interventions (19).

Fasting status can significantly affect triglyceride concentrations; higher results are observed from samples collected post-prandial compared to those obtained while fasting. It is, therefore, recommended when possible that an indication of fasting status be included with triglyceride test results. A comment suggesting repeat assessment after fasting is reasonable to include when non- fasting TG are >400 mg/dL (20).

Most widely used methods to quantify triglycerides start by enzymatically hydrolyzing triglycerides to fatty acids and glycerol, which is quantified. Free glycerol in most specimens is typically less than 1 mg/dL and therefore does not usually affect the TG result. Patients with deficiencies in glycerol metabolism, diabetes mellitus, or chronic kidney disease can have falsely high TG results (21).

Total Cholesterol. TC represents cholesterol from all lipoprotein particles. It includes cholesterol on chylomicrons and their remnants, VLDL, intermediate-density lipoproteins (IDL), LDL, HDL, Lp(a), and lipoprotein X (LpX). TC is minimally influenced by fasting status. The pooled cohort equation endorsed by the US multi-society guidelines on ASCVD risk assessment

TABLE 3. Maximal mean postprandial change in lipid and lipoproteins.

Measure	Pediatrics[a]	Adults[c]
Triglycerides	Increase 0–10 mg/dL	Men: increase 15–30 mg/dL Women: increase 0–20 mg/dL[d]
Total cholesterol	No change	Decrease 3–8 mg/dL
HDL cholesterol	No change	No change
Non-HDL cholesterol	No change	Decrease 3–8 mg/dL
LDL cholesterol	Increase <7 mg/dL[b]	Decrease 3–6 mg/dL[b]
Apolipoprotein B	Not reported	Decrease <5 mg/dL

[a]Based on lipid panel data from 12 744 children, age 3 to 17 years (9).
[b]LDL calculated using the Friedewald formula.
[c]Data from general population observational studies including >100 000 subjects (8, 11, 13).
[d]Women have less impact <20 mg/dL (10).

FIG. 1. Example of a basic lipid panel reporting layout in patient chart.

Laboratory Results	Expected Values	1/25/2024 13:22	9/27/2023 8:05
LIPID PANEL			
Cholesterol, Total	<200 mg/dL	156	258^
Cholesterol, HDL	>40 mg/dL	53	50
Cholesterol, LDL, Calculated	<100 mg/dL	84	185^
Cholesterol, Non-HDL, Calculated	<130 mg/dL	104	128
Triglycerides	<150 mg/dL	103	208^
Fasting (8 HR or more)	N/A	No	Yes

uses TC to calculate 10-year ASCVD risk in conjunction with HDL-C to compensate for the fact that some cholesterol in TC is not atherogenic (22). Universal TC screening to identify genetic or lifestyle-related pediatric dyslipidemia is recommended in 9 to 11 year-olds (1, 2).

Reference intervals for total cholesterol are based on ASCVD risk with desirable concentrations set at the 50th percentile (Table 1). Enzymatic assays for TC depend on the oxidation of the hydroxyl group on the A-ring of cholesterol and therefore can also detect other sterols like si¬tosterol. These other sterols, which are obtained from the diet, contribute only a small part of TC but can accumulate in some rare genetic disorders like sitosterolemia (23). Cholesterol can be differentiated from other sterols by the gas chromatography-based reference method for TC (24).

High-Density Lipoprotein Cholesterol. Unlike cholesterol on other lipoprotein fractions, HDL-C is inversely related to ASCVD risk. Individuals with low HDL-C appear to be at increased ASCVD risk but the cause–effect association is not fully understood. Nevertheless, HDL-C is a principal factor in ASCVD risk assessment. In general, higher HDL-C is better, however, recent studies have shown a more complicated U-shape relationship with ASCVD risk when HDL-C >100 mg/dL (25). Above a certain point, higher HDL-C levels may be pro-atherogenic, but this issue is not addressed by current guidelines. Markedly reduced HDL-C in the absence of liver disease suggests familial HDL deficiency such as Tangier disease or lecithin cholesterol acyltransferase (LCAT) deficiency (26).

Until the advent of direct homogeneous assays, HDL-C was quantified by measuring cholesterol in the supernatant after the manual precipitation of apo B lipoproteins. The fully automated direct HDL-C assays have largely replaced the precipitation methods, but direct HDL-C assays, much like direct LDL-C assays, can also suffer selectivity problems when measured in dyslipidemic samples (7). Reference intervals for HDL-C are based on age- and sex-specific 50th percentiles with lower values indicating higher risk (Table 1). HDL-C <15 mg/dL (2.5th percentile) warrants a comment to investigate liver disease or potential genetic dyslipidemia (26).

Non-High Density Lipoprotein Cholesterol. Non-HDL-C encompasses cholesterol in all apo B-containing atherogenic lipoproteins: LDL, IDL, VLDL, Lp(a), and chylomicrons. Initially, non-HDL-C was proposed as an alternative measure of atherogenic cholesterol in cases when elevated TG prevented LDL-C estimation. However, concordance/discordance analyses have shown that non-HDL-C is as good or better at predicting ASCVD events compared with measured or calculated LDL-C in the general population and in patients on statin therapy.

Non-HDL-C is a simple calculation and should be reported in all lipid panels. Clinical practice guide¬lines recommend non-HDL-C values >190 mg/dL as a risk-enhancing factor indicative of primary hypercholesterolemia (1). Guideline-based non-HDL-C reference intervals have been somewhat arbitrarily defined at 30 mg/dL higher than LDL-C goals (Table 1) and may benefit from further validation.

In addition to ASCVD management, non-HDL-C may help identify LpX, an abnormal lipoprotein that is found in patients with cholestatic or obstructive liver disease. LpX also forms in patients with LCAT deficiency and appears to be causally related to the development of end-stage renal disease in this disorder. Cholesterol in LpX contributes to non-HDL-C, and a pattern of acute onset elevated non-HDL-C and low HDL-C in a context of liver disease should prompt further investigation by lipoprotein electrophoresis to identify LpX.

Lipid Panel Points to Remember

- Laboratories should program their LIS to calculate and report non-HDL-C and LDL-C for all lipid panels.
- All clinical methods for TC, TG, and HDL-C should meet total error limits published by the CDC in normal and dyslipidemic samples (Table 2).
- Fasting is not routinely required for lipid panels except in cases of known hypertriglyceridemia.
- Order sets should be created that allow for both fasting and non-fasting lipid collections.
- Fasting status should be documented at time of collection and reported with any TG results.

LDL CHOLESTEROL

What Is the Best Way to Calculate LDL-C?

Until recently, the Friedewald equation was the only calculation widely used for estimating LDL-C (Eq. 2):

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5 \quad (2)$$

The Friedewald equation requires a fasting sample. The term TG/5 (when in mg/dL) provides an estimate of cholesterol contained in VLDL (VLDL-C). Thus, LDL-C is estimated by subtracting HDL-C and VLDL-C from total plasma cholesterol. A well-known limitation of this equation is that with hypertriglyceridemia VLDL-C is overestimated. The original publication set a hypertriglyceridemia limit of TG <400 mg/dL for use of the Friedewald equa¬tion (12); however, modern studies have demonstrated hypertriglyceridemia leads to LDL-C underestimation at TG >200 mg/dL (27, 28).

Given this limitation, other equations have been developed that more accurately calculate LDL-C in samples with much wider ranges of TG concentrations. In 2013, the Martin equation was first described and later it was extended for higher TG samples (16). It is similar to the Friedewald equation but includes a variable denominator used for estimating VLDL-C (Eq. 3).

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/X \quad (3)$$

The "X"-denominators are empirically defined and range from <4 to >11 depending on non-HDL-C and TG. In 2020, the Sampson equation was first described (Eq. 4) (15).

$$\text{LDL-C} = \frac{\text{TC}}{0.948} - \frac{\text{HDL-C}}{0.971} - \left(\frac{\text{TG}}{8.56} + \frac{\text{TG} \times \text{non-HDL-C}}{2140} - \frac{\text{TG}^2}{16100}\right) - 9.44 \quad (4)$$

Like the Friedewald and Martin equations, the Sampson equation estimates LDL-C by subtracting the cholesterol contribution from HDL-C and VLDL-C from TC. Like the Martin equation, the Sampson equation adjusts the estimated VLDL-C using a weighted interaction between non-HDL-C and TG. Additionally, the Sampson equation corrects for the presence of chylomicrons with a negative TG2 factor. It should also be noted that the Friedewald and Sampson equations were derived using the gold-standard beta-quantification LDL-C method as the reference method. The Martin equation was derived using LDL-C measured by vertical autocentrifugation profile as the reference method. Both the Martin and Sampson equations demonstrate improved comparability to measured LDL-C, particularly when LDL-C <70 mg/dL and when TG are elevated up to 800 mg/dL (16, 28, 29).

Based on recent proficiency test surveys, most clinical laboratories are still using the Friedewald equation, even though the Martin and Sampson equations have been shown to be more accurate, particularly on hypertriglyceridemic samples. The Sampson equation is easily implemented in the LIS, it does not require any additional parameters beyond the basic lipid panel, it is in the public domain, does not require a license fee and, when compared to the beta-quantification, it was shown to be the most accurate equation both when TG are high and LDL-C is low (15).

When Should LDL Cholesterol Be Measured and What Are the Limitations?

Many techniques have been developed for the direct measurement of LDL-C; however, the most widely used assay in clinical laboratories is the enzymatic/colorimetric method. The reason these methods are so popular is because they are commercially available as FDA-approved kits that can be loaded on automated analyzers that require minimal technical expertise or sample preparation and provide a rapid turnaround time. However, these assays are not standardized between different manufacturers and in some cases have been shown to be less reliable than calculating LDL-C (30). In one study that evaluated the performance of 7 of these direct methods, all methods failed the NCEP accuracy goals (total error goal of ≤12%) for dyslipidemic samples (7). For this reason, measuring LDL-C by these enzymatic/colorimetric methods is not universally recommended.

Historically, direct LDL-C was recommended in clinical scenarios where calculated LDL-C was less accurate. This included when LDL-C concentrations were <70 mg/dL or in samples with TG ≥400 mg/dL (27, 31). However, the newer calculations (extended Martin or Sampson equations) have greater accuracy for patients with low LDL-C concentrations and can report results down to 20 mg/dL. Furthermore, these equations have a higher tolerance for hypertriglyceridemia up to 800 mg/dL with comparable performance to direct LDL-C methods (15). Considering the reported failure of direct LDL methods to meet recommended total error goals among individuals with dyslipidemia (7), modern LDL-C calculations may negate the need for direct LDL-C entirely.

None of the LDL-C equations (neither the modern equations nor the Friedewald equation) should be used to calculate LDL-C in patients with type III hyperlipidemia. This disorder affects around 0.6% of the general population and can be detected by calculating the VLDL-C to TG ratio (typically >0.30). The remnant lipoproteins formed in this condition contain an abnormal lipid composition in type III hyperlipidemia that cannot be accounted for by any of these equations. A modified form of the Sampson equation that includes apo B as variable has been shown to accurately estimate VLDL-C in type III hyperlipidemia and can be used to make the diagnosis with high sensitivity and specificity compared to ultracentrifugation (32).

Clinically available ultracentrifuge-based methods include the β-quantification reference method and the VAP method. VAP is a proprietary method with purpose-built hardware clinically performed by VAP Diagnostics Laboratory (formerly Atherotec). The β-quantification technique is considered the definitive reference method for measuring LDL-C and is the method used by the CDC for standardizing routine methods used by clinical laboratories. Because the β-quantification method involves an extensive ultra-centrifugation step (approximately 18 h), it is an unattractive option for routine clinical operations. On the other hand, the VAP method, despite being proprietary to a single reference lab, is attractive because it is much quicker, separating all lipoproteins in less than 1 h thanks to its vertical rotor (33). However, VAP also has an important limitation: it is known to be less accurate than β-quantification in hypertriglyceridemic samples, leading to an underestimation of VLDL-C level, which affects LDL-C calculation. This distinction is important for studies evaluating the accuracy of LDL-C calculations in hypertriglyceridemic samples, and why β-quantification is the preferred reference method for these studies (16).

Lipid Panel Points to Remember

- Reporting names for LDL-C should state the method used (e.g., ß-quant, calculated, direct).
- Calculated LDL-C methods should use a modern equation such as the Sampson or extended- Martin, which account for variations in non-HDL-C and TG.
- Calculated LDL-C should have a lower reporting limit to prevent reporting of inappropriate negative results.
- Evaluation of LDL-C methods should use ß-quantification as the reference method.
- The extended-Martin or Sampson equations allow for accurate LDL estimation at TG concentrations up to 800 mg/dL.
- It is reasonable to not use direct homogeneous methods, which do not always meet CDC guidelines for total allowable error, particularly at low LDL-C (<70 mg/dL) or elevated TG (>400 mg/dL).

LIPOPROTEIN(A)

What Is Lp(a)?

Lp(a) is an apo B-containing atherogenic lipoprotein that has a size, density, and structure that is very similar to LDL. The particle contains the covalent addition of apolipoprotein(a) [apo(a)] to apo B. The apo(a) protein is comprised of a series of Kringle domains including a variable number of type IV2 (KIV2) repeats followed by an inactive plasminogen-like protease domain. Many apo(a) alleles are present in the population differing in the number of KIV2 domain repeats (from 3 to >40), which result in variable lengths of the apo(a) protein and the subsequent size of the Lp(a) lipoprotein particles. Individuals expressing small apo(a) isoforms tend to have higher circulating concentrations compared to those expressing large isoforms. The population concentration of Lp(a) is skewed such that most individuals express low concentrations, while a minority of individuals express very high concentrations of Lp(a). High circulating concentrations of Lp(a) are associated with an increased risk of atherosclerosis and atherosclerotic cardiovascular events, as well as with calcific aortic stenosis.

How to Measure Lp(a)?

Traditionally, the concentration of circulating Lp(a) has been measured using immunoassays, employing antibodies specific for the apo(a) protein found in the particle. This allows for specific measurement of the concentration of this lipoprotein separate from all others in circulation. However, given the heterogeneity of Lp(a) size in the population, immunoassays using polyclonal antibodies with multiple binding sites per particle and optical interference-type detection mechanisms that can be altered by the size of the particle being measured suffer from inaccuracies in measuring particle concentration. While a reference standard exists for manufacturer calibration, commercially available assay results are not harmonized. Historically, immunoassays

have been calibrated in mass units (mg/dL) and most Lp(a) risk cutpoints were derived from population studies measured using mass assays. More recently, assays with assigned calibrators in molar units have been developed and have been shown to be slightly more accurate than mass assays; however, none are currently FDA-approved in the United States. Importantly, it is not recommended to convert mass units to molar units because of the added inaccuracy those calculations contribute to the result. Clinical cutpoints for the Lp(a) test are another gray area. Statistically significant elevated ASCVD risk has been shown above 30 mg/dL, but clinical cutpoints in guidelines have suggested that clinically relevant increased risk starts at an Lp(a) concentration of 50 mg/dL or greater. Those risk cutpoints are higher (typically 75 nmol/L or 125 nmol/L, respectively) when using the molar units assay (3, 34).

Does Lp(a) Influence the Results of the Basic Lipid Panel?

The basic lipid panel will not be useful to identify individuals that express high concentrations of Lp(a). However, because Lp(a) is the same size and density as LDL-C, the cholesterol carried in Lp(a) will be reported as part of the LDL-C measurement in nearly all methods (calculated, direct homogenous methods, and β-quantification). This can cause diagnostic confusion for physicians when investigating the etiology of dyslipidemia. Treatment plans, treatment goals, and cascade testing of family members may all be greatly influenced by knowing that a significant portion of reported LDL-C is coming from Lp(a). Testing for Lp(a)-cholesterol is not routinely available given the difficulty in separating LDL from Lp(a) but can be ordered from reference laboratories.

When Should Lp(a) Be Ordered and How Can It Be Treated?

Guidelines vary on when Lp(a) immunoassay testing should be ordered. European guidelines recommend that Lp(a) be checked at least once in everyone (35). The National Lipid Association has recommended that measurement would be reasonable in individuals with premature atherosclerosis, with a family history of premature atherosclerosis, with a family history of elevated Lp(a), with very high LDL-C levels or familial hypercholesterolemia, and individuals at very high ASCVD risk (34). It is generally recommended that patients with elevated Lp(a) be treated more aggressively by addressing their other risk factors, including elevated LDL-C. Similarly, the US multi-society guidelines include the measurement of Lp(a) as a risk-enhancing factor appropriate in patients at intermediate risk or with significant family history (1).

Lp(a) is genetically determined and circulating concentrations can increase in childhood but do not change much throughout adulthood. Traditional ASCVD risk-lowering lifestyle changes, such as improved diet and increased exercise, do not alter Lp(a) concentrations. Pharmacotherapies, such as statins and ezetimibe, are relatively ineffective and may raise Lp(a) to a small extent in some individuals. Niacin and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors lower Lp(a) by about 25%, but outcome studies looking at cardiovascular events in high Lp(a) expressers have shown mixed results. Niacin added to effective statin therapy does not provide additional cardiovascular outcome benefit (36). However, subgroup analyses of patients with increased Lp(a) in the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial showed improved outcomes with PCSK9 inhibition (37). There are also several new drugs in development that markedly lower Lp(a) by targeting the expression of apo(a), but they are still awaiting cardiovascular outcomes trial data to determine whether they will lower ASCVD events (38).

How Should Lp(a) Be Reported?

Lp(a) should be reported in molar units when possible. However, given the lack of FDA-approved assays, reporting in mass units is still acceptable. Despite known differences in Lp(a) expression in different ethnic groups, and poor standardization and harmonization of methods, multiple societies have endorsed specific clinical decision points. Flagging of results should start at 30 mg/dL when using mass assays or 75 nmol/L when using molar assays to be consistent with the 2018 American Heart Association multi-society guideline recommendations (1). Clinical decision limits used by physicians may vary based on the patient history and risk factor profile.

Lipoprotein(a) Points to Remember

- Lp(a) testing should be performed using an immunoassay that is minimally susceptible to inaccuracies caused by Lp(a) isoform size.
- Lp(a) assays calibrated in molar units are most accurate, but traditional assays calibrated in mass units (mg/dL) are still acceptable.
- Mathematical conversion of results generated using mass-based calibration to molar units should not be performed.
- Flagging of results should start at ≥ 30 mg/dL if using as assay calibrated to mass units or ≥75 nmol/L if calibrated in molar units; however, patient care decisions may use different clinical cutpoints.

APOLIPOPROTEIN B

What Is apo B?

Apolipoprotein B is the core structural lipoprotein of all non-HDL lipoproteins. It is present in chylomicrons and VLDL, which are packaged and secreted with exactly one copy of apo B carried through the entire lipoprotein metabolism pathway. Thus, the

concentration of apo B is directly proportional to the sum of all chylomicrons, VLDL, IDL, Lp(a), and LDL lipoprotein particles present. In most situations, LDL is the most prevalent non-HDL lipoprotein and as such apo B is highly correlated with both non-HDL-C and LDL-C. Despite this correlation, there is great variation in the particle size and lipid content that can result in the same level of LDL-C or non-HDL-C for any given apo B level. As a result, there can be discrepancies in risk classification when comparing these 3 measures.

When Should apo B Be Ordered?

Currently, most relevant societies recommend apo B as a useful biomarker of lipid-derived ASCVD risk but not in the initial screening of ASCVD risk (1, 3, 35). There is growing awareness that apo B may be superior to LDL-C and non-HDL-C as a univariate biomarker of ASCVD risk (1). apo B is especially preferred when TG are elevated, as elevated TG interfere with both estimated and direct homogeneous LDL-C methods. apo B does not have the same limitation as LDL-C, which typically decreases when TG levels rise, due to the production of small dense LDL particles that contain less cholesterol. This phenomenon likely explains why apo B is superior to LDL-C in predicting ASCVD risk when the 2 tests are discordant, which often occurs in diabetic and obese patients. apo B also likely accounts for much of the residual ASCVD risk after lipid-lowering therapy, which typically lowers large LDL particles more than smaller LDL subspecies and hence leads to greater lowering of LDL-C than apo B. Treatment goals based on apo B may, therefore, be better for monitoring lipid-lowering therapy, but at this time apo B is mostly recommended as a risk enhancer test for patients at intermediate 10-year risk.

How Should apo B Be Reported?

The IFCC in collaboration with the WHO maintains a standardized apo B reference material for vendor calibration of clinical methods. A new mass spectrometry reference method is being established for apo B, which will likely improve its standardization compared to LDL-C (39). The primary indication of apo B remains ASCVD risk assessment and as such it should be reported in a context of desirable vs at-risk concentrations. apo B percentiles include 65 mg/dL 10th, 100 mg/dL 75th, and 150 mg/dL 95th. A percentile conversion equation has been described for reporting apo B in LDL-C equivalent units to aid in the education of the value of apo B in ASCVD risk assessment and to allow the use of more familiar LDL-C cutpoints for managing patients.

apo B Points to Remember

- apo B is present on all atherogenic lipoproteins as a single copy and thus provides an in¬tegrated measure of ASCVD risk.
- apo B can be accurately measured by routine clinical methods and its standardization
- will likely further improve with the development of a new reference method.
- When discordant with LDL-C or non-HDL-C, apo B has been shown to be a better predictor of ASCVD events.
- Although not uniformly endorsed by all guidelines for initial risk assessment, greater use of apo B is warranted based on current evidence, particularly as a risk-enhancing factor when monitoring lipid-lowering therapy.

APOLIPOPROTEIN A-I

apolipoprotein A-I (apo AI) is the primary structural lipoprotein for HDL. Some studies have shown that blood concentrations of apo AI and the ratio apo B/apo AI can be predictive of atherosclerosis risk. However, apo AI clinical testing is not widely available, no consensus ASCVD risk thresholds have been established, and no clinical guidelines have endorsed its routine use. Interestingly, the most prominent and growing use of apo AI is as a biomarker of hepatocellular synthesis and functional capacity in liver disease (40).

Apolipoprotein A-I Points to Remember

- apo AI methods are not harmonized or standardized.
- Routine apo AI testing is not recommended.

POINT-OF-CARE LIPID TESTING

What Are the Indications for Point-of-Care Lipid Testing?

Point-of-care testing (POCT) lipid methods have the advantage of short turnaround time and the use of whole blood as an acceptable specimen, which is especially helpful in the outpatient and field settings. Furthermore, POCT lipid testing enables the rapid communication of test results to patients. POCT tests should follow the same certification criteria from the Cholesterol Reference Method Laboratory Network (CRMLN) as serum/plasma-based lipid tests.

What Are the Limitations of Point-of-Care Lipid Testing?

Major limitations include higher imprecision compared to standard chemistry analyzers and narrow linearity ranges, which may result in the need for repeat testing on another analyzer. Another limitation is that typically only tests in the lipid panel are available for POCT testing and it can be challenging to report LDL-C results using one of the newer equations. Lastly, when using fingerstick whole blood, interferences from topical skin lotions can cause false results.

Point-of-Care Lipids Points to Remember

- POCT lipid methods should be held to the same NCEP and CRMLN performance metrics as other methods.
- POCT reporting should clearly describe the method, including any calculated LDL-C.

LDL SUBFRACTIONS

LDL is a heterogenous collection of different size lipoprotein particles with diameters between 19 and 22 nm. It is also heterogenous in terms of its density, protein content, and lipid composition. Using a variety of different techniques, such as those based on nuclear magnetic resonance (NMR), electrophoresis, density gradient centrifugation, gel filtration, and ion mobility, it is possible to separately measure the different subfractions of LDL. Because these different tests rely on different physical principles, the results of the different LDL subfraction tests are not interchangeable and often have different nomenclature (39). Some of the LDL subfractions like small dense LDL may be better predictors of ASCVD risk than total LDL-C. A fully automated direct assay for small dense LDL-C is available (41), and more recently, an equation estimating small dense LDL-C from lipid panel test results was developed (32). LDL subfraction testing, however, is not recommended by any current guidelines.

TABLE 4. Summary of recommendations to clinical laboratories and clinicians.

Number	Recommendation	Laboratories	Clinicians
1	Standard lipid panels should report 3 measured values: TC, TG, HDL-C, and 2 calculated parameters: LDL-C and non-HDL-C.	✓	✓
2	Test names for the lipid panel and its components should indicate the specific analyte being measured, the sample obtained, and method used when appropriate.	✓	
3	There now exists a wide variety of LDL-C methods and calculations in routine use, so it is best practice to state either calculated or measured LDL-C in the reporting name. The LDL-C calculation method should also be included in the report comment or laboratory test catalog.	✓	
4	Lipids are best interpreted in a context of "desirable" vs "increased risk," typically defined between the 50th and 75th percentiles of healthy populations.	✓	✓
5	Adult patients with hypertriglyceridemia and pediatric patients with elevated non-HDL-C should be retested on a fasting sample.		✓
6	Order sets should allow for both fasting and non-fasting lipid panels, and fasting status should be documented at time of collection and indicated in the result report.	✓	
7	A comment suggesting repeat assessment after fasting is reasonable to include when non-fasting TG are >400 mg/dL.	✓	
8	Universal TC screening to identify genetic or lifestyle-related pediatric dyslipidemia is recommended in 9–11 year-olds.		✓
9	HDL-C <15 mg/dL (2.5th percentile) warrants a comment to investigate liver disease or potential genetic dyslipidemia.	✓	
10	Cholesterol in LpX contributes to non-HDL-C, and a pattern of acute onset elevated non-HDL-C and low HDL-C in a context of liver disease should prompt further investigation by lipoprotein electrophoresis to identify LpX.		✓
11	Switch from the Friedewald equation that calculates LDL-C to either the Sampson or Martin equations. Both the Martin and Sampson equations demonstrate improved comparability to measured LDL-C, particularly when LDL-C <70 mg/dL and when TG are elevated up to 800 mg/dL.	✓	✓
12	Measuring LDL-C by direct homogeneous methods is not universally recommended. Laboratories calculating LDL-C by the extended Martin or Sampson equations have reduced need for direct LDL-C because of improved estimation performance in samples with low LDL-C or high triglycerides (up to 800 mg/dL). Addressing triglyceride levels when >800 mg/dL should be the immediate focus before considering LDL-C management.	✓	

TABLE 4. Continued			
13	None of the LDL-C equations (neither the modern equations nor the Friedewald equation) should be used to calculate LDL-C in patients with type III hyperlipidemia. Laboratories are encouraged to add a comment in patient reports highlighting this drawback.	✓	✓
14	If LDL-C is necessary in patients with type III hyperlipidemia or TG >800 mg/dL, then β-quantification is the preferred method.	✓	✓
15	Lp(a) should be reported in molar units, when possible, but it is not recommended to mathematically convert mass units to molar units because of the added inaccuracy those calculations contribute to the result.	✓	
16	Lp(a) results >30 mg/dL when using mass assays or >75 nmol/L when using molar assays should be flagged as high. Clinical decision limits used by clinicians may vary based on the patient history and risk factor profile.	✓	✓
17	Measurement of Lp(a) would be reasonable in individuals with premature atherosclerosis, with a family history of premature atherosclerosis, with a family history of elevated Lp(a), with very high LDL-C levels or familial hypercholesterolemia, and individuals at very high ASCVD risk. European guidelines recommend measuring Lp(a) at least once in everyone.		✓
18	Most relevant societies recommend apo B as useful biomarker of lipid-derived ASCVD risk but not in the initial screening of ASCVD risk. apo B may be superior to LDL-C and non-HDL-C as a univariate biomarker of ASCVD risk. apo B is especially preferred when TG are elevated, as elevated TG interfere with both estimated and direct homogeneous LDL-C methods. At this time, apo B is mostly recommended as a risk enhancer test for patients at intermediate 10-year risk.		✓
19	Warning when using POCT lipid methods: when using fingerstick whole blood, interferences from topical skin lotions can cause false results.	✓	✓
20	Routine use of LDL subfraction testing is not recommended by any current guidelines.	✓	✓

LDL Subfraction Points to Remember

- LDL subfraction methods are not harmonized or standardized.
- Routine LDL subfraction testing is not recommended.

SUMMARY

In conclusion, the recommendations outlined in this document provide guidance for standardizing lipid reports and improving the interpretation of li¬pid panel results (Table 4). By emphasizing the reporting of specific measured values and calculated parameters, indicating test names with clarity, and specifying LDL-C methods, healthcare professionals can achieve greater consistency and accuracy in lipid reporting. We reinforce the importance of interpreting lipids within the context of healthy population percentiles and underscore the need for fasting samples only when adjusting medication dosage or in patients with hypertriglyceridemia. The recommendations also address the reporting and interpretation of Lp(a) results, the use of apo B as a biomarker, and the precautions necessary when using POCT lipid methods. It is our hope that these recommendations and subsequent revisions serve as a comprehensive resource to support clinicians and laboratories in lipid assessment and enhancing patient care.

Nonstandard Abbreviations: Non-HDL-C, non-high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); apo B, apolipoprotein B; apo(a), apolipoprotein(a); ASCVD, atherosclerotic cardiovascular disease; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; NCEP, National Cholesterol Education Program; VLDL, very-low-density lipoprotein; LpX, lipoprotein X; VLDL-C, very-low-density lipoprotein cholesterol; apo AI, apolipoprotein A-I; POCT, point-of-care testing.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

Jeffrey Meeusen (Conceptualization-Equal, Data curation-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Leslie Donato (Conceptualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Anne Goldberg (Conceptualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Jing Cao (Conceptualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Joe El-Khoury (Conceptualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), and Alan Remaley (Conceptualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal)

Authors' Disclosures or Potential Conflicts of Interest:
Upon manuscript submission, all authors completed the author disclosure form.

Research Funding: None declared.

Disclosures: A. Goldberg disclosed clinical trials—payment to institution from Novartis, Amgen, IONIS, Regeneron, Arrowhead, New Amsterdam, Sanofi, Bio89, and Esperion. A. Goldberg received consulting fees from New Amsterdam; honoraria from National Lipid Association, American College of Cardiology, and Preventive Cardiology Nurses Association; free registration to American Heart Association, Endocrine Society, and American College of Cardiology meetings when moderating or presenting; travel support from Austrian American Foundation; and medical writing assistance for group manuscripts from Esperion. A. Goldberg participated in advisory board meetings for AKCEA and Regeneron, data monitoring board for Lilly, and clinical trial steering committee for New Amsterdam, is country lead investigator for IONIS, and holds leadership roles with National Lipid Association and Foundation of the National Lipid Association. L. Donato received honoraria from Novartis and Helena. J.M. El-Khoury disclosed research grants to institution from Siemens Healthineers and Bioporto; consulting fees from Siemens Healthineers; honoraria, travel, and lodging support from ADLM; blood tubes for unrelated study from Becton-Dickinson; and a mass spectrometer for evaluation from Ionsense (now part of Bruker). J.M. El-Khoury serves on the Board of Directors of ADLM and is Associate Editor for Clinical Chemistry.

Role of Sponsor: No sponsor was declared.

REFERENCES

1. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ ABC/ACPM/ADA/ AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation 2019;139: e1046–81.
2. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011;128(Suppl 5):S213–56.
3. Wilson PWF, Jacobson TA, Martin SS, Jackson EJ, Le NA, Davidson MH, et al. Lipid measurements in the management of cardiovascular diseases: practical recommendations a scientific statement from the national lipid association writing group. J Clin Lipidol 2021;15:629–48.
4. White-Al Habeeb NMA, Higgins V, Venner AA, Bailey D, Beriault DR, Collier C, et al. Canadian society of clinical chemists harmonized clinical laboratory lipid reporting recommendations on the basis of the 2021 Canadian cardiovascular society lipid guidelines. Can J Cardiol 2022; 38:1180–8.
5. Vesper HW, Wilson PWF, Rifai N. A message from the laboratory community to the National Cholesterol Education Program Adult Treatment Panel IV. Clin Chem 2012;58:523–7.
6. Centers for Disease Control and Prevention. CRMLN: Cholesterol Reference Method Laboratory Network. http://www.cdc.gov/labstandards/crmln.html/(Accessed November 2020).
7. Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, Dziekonski A, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 2010;56:977–86.
8. Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. Clin Chem 2011; 57:482–9.
9. Steiner MJ, Skinner AC, Perrin EM. Fasting might not be necessary before lipid screening: a nationally representative cross-sectional study. Pediatrics 2011; 128:463–70.
10. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation 2008;118: 993–1001.
11. Sidhu D, Naugler C. Fasting time and lipid levels in a community-based population: a cross-sectional study. Arch Intern Med 2012;172:1707–10.
12. 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.
13. Mora S, Chang CL, Moorthy MV, Sever PS. Association of nonfasting vs fasting lipid levels with risk of major coronary events in the Anglo-Scandinavian cardiac outcomes trial-lipid lowering arm. JAMA Intern Med 2019; 179:898–905.
14. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 2008;118:2047–56.

15. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, et al. A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. JAMA Cardiol 2020;5:540–8.

16. Sajja A, Park J, Sathiyakumar V, Varghese B, Pallazola VA, Marvel FA, et al. Comparison of methods to estimate low-density lipoprotein cholesterol in patients with high triglyceride levels. JAMA Netw Open 2021;4:e2128817.

17. Daniels SR, Gidding SS, de Ferranti SD; National Lipid Association Expert Panel on Familial Hypercholesterolemia. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol 2011;5:S30–7.

18. Gill PK, Dron JS, Hegele RA. Genetics of hypertriglyceridemia and atherosclerosis. Curr Opin Cardiol 2021;36:264–71.

19. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292–333.

20. Stone NJ, Grundy SM. The 2018 AHA/ACC/multi-society cholesterol guidelines: looking at past, present and future. Prog Cardiovasc Dis 2019;62:375–83.

21. Backes JM, Dayspring T, Moriarty PM. Pseudohypertriglyceridemia-Raising clinical awareness. J Clin Lipidol 2019;13:855–6.

22. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB Sr, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63:2935–59.

23. Tada H, Nohara A, Inazu A, Sakuma N, Mabuchi H, Kawashiri MA. Sitosterolemia, hypercholesterolemia, and coronary artery disease. J Atheroscler Thromb 2018;25: 783–9.

24. Cohen A, Hertz HS, Mandel J, Paule RC, Schaffer R, Sniegoski LT, et al. Total serum cholesterol by isotope dilution/mass spectrometry: a candidate definitive method. Clin Chem 1980;26:854–60.

25. Yang HS, Jeong HJ, Kim H, Hwang HK, Hur M, Lee S. Sex-specific U-shaped relationships between high-density lipoprotein cholesterol levels and 10-year major adverse cardiovascular events: a nationwide cohort study of 5.7 million South Koreans. Ann Lab Med 2022;42: 415–27.

26. Bonilha I, Luchiari B, Nadruz W, Sposito AC. Very low HDL levels: clinical assessment and management. Arch Endocrinol Metab 2023;67:3–18.

27. Meeusen JW, Snozek CL, Baumann NA, Jaffe AS, Saenger AK. Reliability of calculated low-density lipoprotein cholesterol. Am J Cardiol 2015;116:538–40.

28. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA 2013;310:2061–8.

29. 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.

30. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. Clin Chem 2009;55:888–94.

31. Whelton SP, Meeusen JW, Donato LJ, Jaffe AS, Saenger A, Sokoll LJ, et al. Evaluating the atherogenic burden of individuals with a Friedewald-estimated low-density lipoprotein cholesterol <70 mg/dL compared with a novel low-density lipoprotein estimation method. J Clin Lipidol 2017;11:1065–72.

32. Sampson M, Wolska A, Meeusen JW, Donato LJ, Jaffe AS, Remaley AT. Identification of dysbetalipoproteinemia by an enhanced Sampson-NIH equation for very low-density lipoprotein-cholesterol. Front Genet 2022;13: 935257.

33. Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. Clin Lab Med 2006;26:787–802.

34. Wilson DP, Jacobson TA, Jones PH, Koschinsky ML, McNeal CJ, Nordestgaard BG, Orringer CE. Use of lipoprotein(a) in clinical practice: a biomarker whose time has come. A scientific statement from the National Lipid Association. J Clin Lipidol 2019;13:374–92.

35. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;41: 111–88.

36. AIM-HIGH Investigators; Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 2011;365: 2255–67.

37. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, et al. Lipoprotein(a), PCSK9 inhibition, and cardiovascular risk. Circulation 2019;139: 1483–92.

38. Sosnowska B, Surma S, Banach M. Targeted treatment against lipoprotein (a): the coming breakthrough in lipid lowering therapy. Pharmaceuticals (Basel) 2022;15:1573.

39. Delatour V, Clouet-Foraison N, Gaie-Levrel F, Marcovina SM, Hoofnagle AN, Kuklenyik Z, et al. Comparability of lipoprotein particle number concentrations across ES-DMA, NMR, LC-MS/MS, immunonephelometry, and VAP: in search of a candidate reference measurement procedure for apoB and non-HDL-P standardization. Clin Chem 2018;64:1485–95.

40. Gurbuz B, Guldiken N, Reuken P, Fu L, Remih K, Preisinger C, et al. Biomarkers of hepatocellular synthesis in patients with decompensated cirrhosis. Hepatol Int 2023;17:698–708.

41. Tsai MY, Steffen BT, Guan W, McClelland RL, Warnick R, McConnell J, et al. New automated assay of small dense low-density lipoprotein cholesterol identifies risk of coronary heart disease: the Multi-ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol 2014;34: 196–201.