

ORIGINAL ARTICLE

Revisiting the Triglyceride Threshold for LDL-C Calculation Considering Measurement Uncertainty

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ABSTRACT

Background: Accurate estimation of LDL cholesterol (LDL-C) is essential for cardiovascular risk assessment and treatment. The widely-used Friedewald equation loses reliability at higher triglyceride (TG) levels, particularly near the 400 mg/dL (4.5 mmol/L) threshold. This study aimed to evaluate the measurement uncertainty (MU) of TG, total cholesterol (CHOL), HDL cholesterol (HDL-C), directly-measured LDL-C, and Friedewald-calculated LDL-C using the Nordtest guideline and aimed to determine whether the conventional TG threshold remains analytically appropriate when MU is considered.

Methods: We retrospectively analyzed 12-month internal quality control (IQC) and external quality assessment (EQA) data from the lipid panel performed in a single-center laboratory using the Mindray BS-2000M analyzer. Combined and expanded MU values were calculated for each analyte and compared to maximum allowable uncertainty (MAU) targets. The impact of TG MU on the Friedewald equation's TG threshold was assessed using routine patient data from February 2024 through January 2025.

Results: MU values for TG (8.4%) and direct LDL-C (6.2%) were within their MAU targets, while those for HDL-C (8.7%) and CHOL (7.7%) exceeded their respective thresholds. The calculated LDL-C showed a higher MU (13.3%), largely influenced by TG and HDL-C variability. Based on the 8.4% MU of TG, a revised upper threshold of 369 mg/dL (4.2 mmol/L) was proposed. Out of 122,639 patient results, 848 (0.7%) had TG levels between 369 - 400 mg/dL, indicating potential LDL-C misclassification.

Conclusions: Measurement uncertainty substantially impacts the reliability of calculated LDL-C near the TG threshold. Clinical laboratories should consider incorporating MU into LDL-C interpretation and re-evaluating TG limits to improve risk stratification and treatment accuracy.

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KEYWORDS

LDL cholesterol, triglycerides, hyperlipidemias, measurement uncertainty, clinical laboratory techniques

INTRODUCTION

Cardiovascular disease is the leading cause of death and disability worldwide, with atherosclerotic cardiovascular disease (ASCVD) accounting for most cases. Among lipid-related risk factors, low-density lipoprotein cholesterol (LDL-C) is firmly established as a key contributor to the initiation and progression of atherosclerosis. Large-scale genetic and epidemiological studies have

consistently demonstrated that elevated LDL-C levels are directly associated with increased ASCVD risk and cardiovascular mortality [1-3]. LDL-C is a major component of all apolipoprotein B (apoB)-containing lipoproteins, including very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) [Lp(a)], all of which are considered atherogenic [4]. However, LDL-C remains the primary target of lipid-lowering therapies and the cornerstone of ASCVD risk assessment.

The 2019 European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines recommend LDL-C treatment targets based on total cardiovascular risk, ranging from < 1.4 mmol/L (< 55 mg/dL) for very high-risk patients to < 3.0 mmol/L (< 116 mg/dL) for those at low risk [5]. Meta-analyses by the Cholesterol Treatment Trialists' (CTT) Collaboration have shown that each 1 mmol/L (approximately 40 mg/dL) reduction in LDL-C corresponds to a 20% relative reduction in cardiovascular events [6]. Furthermore, statin therapy has been proven to reduce ASCVD risk in proportion to the absolute decrease in LDL-C levels [7,8]. These findings highlight the importance of accurate LDL-C measurement in clinical decision-making.

LDL-C levels can be determined either by direct measurement or estimation from other lipid profile components. The gold standard method, β -quantification via ultracentrifugation, is highly accurate but impractical for routine clinical use due to its complexity, cost, and sample volume requirements [9,10]. As an alternative, many laboratories rely on automated homogeneous assays, particularly when TG levels exceed 400 mg/dL (4.5 mmol/L) [11]. While direct methods offer reasonable accuracy, limitations such as poor standardization, variable analytical performance, and increased cost often lead laboratories to favor calculated LDL-C methods [12,13].

The most widely-used calculation method is the Friedewald formula, which estimates LDL-C as [14]:

Eq. 1:

$\text{LDL-C (mg/dL)} = (\text{CHOL}) - (\text{HDL-C}) - (\text{TG}/5)$ (for mmol/L, TG/2.2 is used).

This formula assumes a constant TG-to-VLDL-C ratio of 5:1 under fasting conditions and is generally valid when TG levels are below 400 mg/dL (4.5 mmol/L). However, it has well-documented limitations. Its accuracy decreases significantly when TG levels exceed 200 mg/dL (2.3 mmol/L), particularly in non-fasting states, where postprandial lipemia elevates TG-rich lipoproteins such as chylomicrons and their remnants, distorting the underlying assumptions of the equation [11,14]. In individuals with hypertriglyceridemia or rare dyslipidemias such as type III hyperlipoproteinemia, where cholesterol-rich IDL and VLDL remnants predominate, the fixed ratio becomes biologically invalid. These limitations are particularly concerning considering the in-

creasing use of non-fasting lipid panels and the high prevalence of adults with TG > 150 mg/dL (1.7 mmol/L) [15,16]. In such cases, applying the Friedewald formula without considering individual context may lead to LDL-C underestimation and potential misclassification of cardiovascular risk [14].

Since lipid disorders are independent risk factors for ASCVD, clinicians rely on lipid profile measurements to be both precise and reliable. However, even with validated analytical methods, repeated measurements of the same analyte can yield slightly different results due to inherent variation in the measurement process. This variation is expressed as measurement uncertainty (MU) - a parameter that quantifies the expected range within which the true value of a result lies [17]. MU accounts for both imprecision and bias and provides insight into the level of confidence one can place at a reported value [17,18].

In clinical practice, MU becomes particularly relevant when test results approach clinical decision thresholds, where even small analytical variations can affect treatment decisions. For example, an LDL-C value just below a therapeutic target may, when MU is considered, fall outside that range - altering risk classification or therapy initiation [19-21]. Despite its importance, MU remains underreported and underutilized in routine interpretation of calculated lipid parameters. The ISO 15189 standard requires laboratories to determine and document MU for each quantitative test method [22], yet its integration into LDL-C interpretation, particularly for calculated values, is limited.

In this study, we aimed to assess the impact of MU - particularly that of triglyceride measurements - on the reliability of LDL-C estimation using the Friedewald equation. By using the Nordtest guideline, we evaluated whether the conventional TG threshold of 400 mg/dL (4.5 mmol/L) remains analytically appropriate and we examined the potential clinical implications of incorporating MU into lipid interpretation.

MATERIALS AND METHODS

Our study is a retrospective, single-center study conducted using 12-month internal quality control (IQC) and external quality assessment (EQA) data from the Medical Biochemistry Laboratory of Istanbul Gaziosmanpaşa Training and Research Hospital for the period between February 2024 and January 2025. The study was approved by the Clinical Research Ethics Committee of Gaziosmanpaşa Training and Research Hospital (decision date and number: 01-22-2025/14) and was conducted in accordance with the principles of the Helsinki Declaration.

We calculated MU values for each analyte involved in Friedewald formula (i.e., TG, HDL-C, CHOL), calculated LDL-C, and directly measured LDL-C. Moreover, we retrospectively examined the patient TG results (i.e., TG ≤ 400 mg/dL) for assessing the TG cutoff reliability.

Patient results with TG test values above 400 mg/dL and/or incomplete lipid profiles were excluded from this part of the study.

For the calculation of combined standard uncertainties and expanded uncertainties, we used the Nordtest guidelines [23], which provide a practical, understandable, and universally applicable approach to measurement uncertainty (MU) estimation. The definitions of the lipid panel analytes for which MU values were calculated are provided in Table 1. These lipid panel analytes were measured in the Medical Biochemistry Laboratory of Gaziosmanpaşa Training and Research Hospital using the Mindray BS-2000M analyzer (Mindray, Shenzhen, China) and the manufacturer's reagent kits. IQC was performed with manufacturers original control samples (ClinChem Multi Control Level 1 and 2). EQA was performed with QualiCont EQA Scheme (QualiCont Non-profit Kft., Szeged, Hungary).

Nordtest measurement uncertainty calculations

Step 1: Calculation of within-laboratory reproducibility (Rw)

To calculate the within-laboratory reproducibility (Rw), we used the 12-month coefficient of variation (%CV) values of IQC materials at normal and pathological levels. The %CV was calculated using the standard deviation (SD) and mean ($\bar{x}$) obtained from the dataset, following the formula:

Eq. 2:

$$CV\% = \left(\frac{SD}{\bar{x}}\right) \times 100$$

The within-laboratory reproducibility was calculated using the following equation:

Eq. 3:

$$Rw = \sqrt{(\text{Level 1 CV})^2 + (\text{Level 2 CV})^2} / 2$$

The measurement uncertainty contribution from reproducibility was then determined as follows:

Eq. 4:

$$u(Rw) = Rw/2$$

Step 2: Calculation of bias-related uncertainty (u(bias))

For the estimation of the uncertainty component arising from potential methodological or laboratory bias, we utilized the root mean squares of bias (RMS bias) and the uncertainty component of the certified or nominal value ($u(\text{Cref})$) obtained from 12-month QualiCont EQA data. The RMS bias was calculated as follows:

Eq. 5:

$$\text{Intra group bias} = \sqrt{(\sum \text{Intra group bias})^2 / N}$$

Eq. 6:

$$\text{Inter group bias} = \sqrt{(\sum \text{Inter group bias})^2 / N}$$

N = number of participating EQA schemes.

Eq. 7:

$$\text{RMS bias} = \sqrt{((\text{Intra group bias})^2 + (\text{Inter group bias})^2) / 2}$$

The $u(\text{Cref})$ value was determined using the following equations:

Eq. 8:

$$u(\text{Cref}_i) = CV\% / \sqrt{n}$$

Eq. 9:

$$u(\text{Cref}) = \sum u(\text{Cref}_i) / N$$

$u(\text{Cref}_i)$: Individual $u(\text{Cref})$ value for each EQA sample assessment:

n_{lab} : Number of laboratories using the same method and the same instrument (peer group participants)

N: number of EQA schemes participated.

By using the calculated RMS bias and $u(\text{Cref})$ values, the bias-related uncertainty component was computed as follows:

Eq. 10:

$$u(\text{bias}) = \sqrt{(\text{RMS bias})^2 + (u(\text{Cref}))^2}$$

Step 3: Calculation of combined standard uncertainty (u_c)

The combined standard uncertainty (u_c) was computed using the following equation, which incorporates the calculated standard uncertainty components:

Eq. 11:

$$u_c = \sqrt{(u(Rw))^2 + (u(\text{bias}))^2}$$

Step 4: Calculation of expanded uncertainty (U)

In the final step, the expanded uncertainty (U) value was determined by multiplying the u_c value by a coverage factor (k), which was rounded to 2 to represent a 95% confidence interval:

Eq. 12:

$$U = u_c \times 2$$

For calculated LDL-C MU estimation, the uncertainty components of the independent variables in the calculation equation were combined using the following formula:

Eq. 13:

$$u_{\text{LDL-C (Calculated)}} = \sqrt{(u(\text{CHOL}))^2 + (u(\text{HDL}))^2 + (u(\text{TG})/5)^2}$$

The expanded measurement uncertainty for LDL-C was then calculated by multiplying the standard uncertainty (u) by the coverage factor (k):

Eq. 14:

$$U_{\text{LDL-C (Calculated)}} = u_{\text{LDL-C (Calculated)}} \times 2$$

To compare the calculated MU values, we selected the maximum expanded allowable measurement uncertainty (MAU) targets from the desirable targets in the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation database [24]. Microsoft Office 365 (Microsoft Excel Software, Microsoft Corporation, US) was used for data calculations and table generation.

RESULTS

In our study, the measurement uncertainty (MU) for triglycerides (TG) was calculated as 8.4%, based on the Nordtest guidelines. The calculated MU value for TG remained within the maximum allowable uncertainty (MAU) threshold of 19.7%. For LDL-C, the measurement uncertainty was determined to be 6.2%, which was also within the MAU limit of 7.7%. For HDL-C, the measurement uncertainty was calculated as 8.7%; however, this value exceeded the MAU threshold of 5.7%. Similarly, for total cholesterol (CHOL), the MU was 7.7%, which also surpassed the MAU limit of 5.2%. In the uncertainty calculation for calculated LDL-C using the Friedewald formula, the MU value was found to be 13.3%. The measurement uncertainty components of the analytes included in the Nordtest-based calculations, along with their respective MAU values, are presented in Table 2.

To ensure the safe use of the Friedewald formula, we considered an 8.4% MU correction for the upper limit of TG. Based on this adjustment, we determined that the reliable upper limit for TG should be 369 mg/dL (4.2 mmol/L). This value was calculated on the assumption that when the $\pm 8.4\%$ MU is applied to 369 mg/dL, the upper bound of the confidence interval reaches approximately 400 mg/dL ($369 \times 1.084 \approx 400$). In other words, a TG measurement of 369 mg/dL represents the highest value at which the true TG concentration is still likely to fall within the clinically accepted threshold. By applying this adjusted cutoff to our dataset, we identified 848 out of 122,639 patients (0.7%) with TG levels between 369 and 400 mg/dL (4.2 - 4.5 mmol/L). The LDL-C values estimated for these patients may have been affected by MU and therefore require cautious interpretation, particularly when close to clinical decision limits.

DISCUSSION

This study evaluated the impact of measurement uncertainty (MU) on the reliability of calculated LDL-C values using the Friedewald equation. Our findings demonstrated that while TG and directly measured LDL-C met their respective maximum allowable uncertainty (MAU) thresholds, HDL-C and CHOL exceeded them. Notably, the MU for calculated LDL-C was 13.3%, more than double the MU observed for direct LDL-C measurement (6.2%). This elevated uncertainty was primarily driven by the bias component ($u(\text{bias})$) derived from external quality assessment (EQA) data, consistent with previous reports by Tan et al. and Nurlu et al., who also identified $u(\text{bias})$ as the largest contributor to overall MU in Nordtest-based calculations [25,26].

By applying the calculated 8.4% MU for TG, we proposed a revised upper threshold of 369 mg/dL (4.2 mmol/L) for reliable use of the Friedewald formula. At this level, the 95% confidence interval for TG remains within clinically acceptable limits. In contrast, a TG result of 400 mg/dL could reflect a true value ranging from 366 to 434 mg/dL due to analytical uncertainty, increasing the risk of LDL-C underestimation. Our retrospective analysis showed that 848 patients (0.7%) in our cohort had TG values within the 369 - 400 mg/dL range, indicating that even a small patient subset could be at risk of misclassification if MU is not considered. The clinical importance of MU has been illustrated in other areas of laboratory medicine. For example, Ustundağ et al. demonstrated how MU influences the interpretation of forensic ethanol results near legal decision thresholds [27], while Kin Tekce et al. showed that incorporating MU into serum creatinine results significantly impacts the diagnosis of acute kidney injury [28]. These studies reinforce the value of integrating MU into clinical decision-making, particularly when test results are near diagnostic or therapeutic cutoffs.

Given the limitations of the Friedewald formula under specific conditions - such as non-fasting states, moderate-to-severe hypertriglyceridemia, or rare lipid disorders like type III hyperlipoproteinemia - several alternative LDL-C estimation methods have been developed. Among these, the Martin-Hopkins equation has shown improved accuracy in individuals with LDL-C < 1.8 mmol/L (70 mg/dL) and TG up to 4.5 mmol/L (400 mg/dL). However, its accuracy diminishes beyond this range due to poor correlation with β -quantification [29, 30]. Similarly, the Sampson equation, developed using β -quantification data from hypertriglyceridemic populations, performs well up to TG levels of 800 mg/dL (9.0 mmol/L) and may offer improved accuracy in normolipidemic individuals [31].

Recognizing these challenges, the EAS and the EFLM have issued consensus recommendations. These advocate for consistency in LDL-C measurement methods throughout treatment, clear communication to clinicians when method changes occur, and preferential use of the

Table 1. Clinical chemistry measurands.

Analyte	Unit	Analytical method	Biological system
Triglyceride (TG)	mg/dL	enzymatic colorimetric method	serum
Total cholesterol (CHOL)	mg/dL	enzymatic colorimetric method	serum
HDL-cholesterol (HDL-C)	mg/dL	homogeneous enzymatic colorimetric method	serum
LDL-cholesterol (LDL-C)	mg/dL	homogeneous enzymatic colorimetric method	serum

Table 2. Measurement uncertainty components of clinical chemistry analytes according to the Nordtest approach.

Analyte	CV ₁ (%)	CV ₂ (%)	u^2R_w	RMS bias ²	$u(\text{Cref})^2$	$u(\text{bias})$	u_c	U	MAU
TG	2.85	2.49	1.90	15.63	0.09	3.96	4.20	8.40	19.7
LDL-C	2.25	2.43	1.37	8.20	0.12	2.88	3.11	6.23	7.7
HDL-C	3.16	3.43	2.70	15.98	0.06	4	4.33	<u>8.66</u>	5.7
CHOL	2.73	2.93	2	12.85	0.08	3.60	3.86	<u>7.73</u>	5.2
Calculated LDL-C	-	-	-	-	-	-	6.64	13.28	-

TG: triglyceride, LDL-C: LDL cholesterol, HDL-C: HDL cholesterol, CHOL: total cholesterol, CV₁: coefficient variation of level 1 control, CV₂: coefficient variation of level 2 control, u^2R_w : standard uncertainty component for the long-term precision obtained from twelve month-internal quality control, RMS bias²: root mean square of biases, $u(\text{Cref})^2$: the uncertainty of nominal values, $u(\text{bias})$: uncertainty component of the bias, u_c : combined standard uncertainty, U : expanded uncertainty, MAU - maximum expanded allowable measurement uncertainty. Bold and underlined values indicate expanded uncertainty exceeding the MAU.

Martin-Hopkins equation in patients with LDL-C < 1.8 mmol/L or TG levels between 175 - 400 mg/dL (2.0 - 4.5 mmol/L). For TG > 4.5 mmol/L (400 mg/dL), direct LDL-C measurement is advised [30,32].

Recent developments in machine learning-based LDL-C estimation also show promise. These models aim to account for complex lipid interactions and may improve accuracy and reduce uncertainty across diverse populations [33,34]. However, regardless of the formula used, it is important to recognize that the MU of calculated LDL-C is inherently affected by the uncertainties of its input variables - namely, CHOL, HDL-C, and TG. Our study confirms that calculated LDL-C carries a significantly higher MU than directly measured LDL-C, underscoring the added value of direct methods in situations where analytical precision is critical.

This study has several limitations. It was conducted in a single-center setting using one analytical platform, which may limit generalizability to other laboratories with different instruments or calibration protocols. MU was calculated using IQC data based on manufacturer-provided control materials rather than third-party reference standards, which could affect comparability with other settings. Additionally, the retrospective nature of the study limited our ability to control patient-level variables such as fasting status, comorbidities, or use of lipid-lowering therapy.

In conclusion, considering the 8.4% MU value for TG,

we determined that the upper TG inclusion limit for the Friedewald equation should be 369 mg/dL rather than 400 mg/dL. Since the accuracy of Friedewald-derived LDL-C estimates is directly dependent on TG levels, it is crucial to ensure the reliability of TG measurements. If TG results are near the equation's upper limit, incorporating MU into test result interpretation could significantly impact the diagnosis and treatment of dyslipidemic patients. We suggest that clinical laboratories should integrate MU assessments when using the Friedewald formula, particularly in patients with borderline TG levels, to improve clinical decision-making.

Ethical Approval Statement:

The research related to human use has been complied with all the relevant national regulations and institutional policies and is in accordance with the tenets of the Helsinki Declaration. It has been approved by the Clinical Research Ethics Committee of Gaziosmanpaşa Training and Research Hospital (decision date and number: 01-22-2025/14).

Declaration of Generative AI in Scientific Writing:

AI-assisted writing support (ChatGPT) was used under author supervision for English language editing.

Declaration of Interest:

The authors declare no conflicts of interest.

References:

1. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017;38:2459-72. (PMID: 28444290)
2. Cholesterol Treatment Trialists' (CTT) Collaborators; Mihaylova B, Emberson J, Blackwell L, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: Meta-analysis of individual data from 27 randomised trials. *Lancet* 2012 Aug 11;380(9841):581-90. (PMID: 22607822)
3. Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts): Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur J Prev Cardiol* 2016 Jul;23(11):NP1-NP96. (PMID: 27353126)
4. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015;161:161-72. (PMID: 25815993)
5. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk [Erratum in *Eur Heart J* 2020 Nov 21; 41(44):4255]. *Eur Heart J* 2020;41(1):111-88. (PMID: 31504418)
6. Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010;376(9753):1670-81. (PMID: 21067804)
7. Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016; 388:2532-61. (PMID: 27616593)
8. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. *N Engl J Med* 2017;376:1713-22. (PMID: 28304224)
9. Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. *Clin Chem* 1995;41(10):1414-20. (PMID: 7586510)
10. Martin SS. Calculating LDL cholesterol in familial combined hyperlipidemia: Out with the old, in with the new? *Atherosclerosis* 2018;277:172-4. (PMID: 30166207)
11. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin Chem* 2002;48(2): 236-54. (PMID: 11805004)
12. Wolska A, Remaley AT. Measuring LDL-cholesterol: what is the best way to do it? *Curr Opin Cardiol* 2020;35(4):405-11. (PMID: 32412961)
13. Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clin Chem* 2010;56(6):977-86. (PMID: 20378768)
14. 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(6): 499-502. (PMID: 4337382)
15. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APHA/ASPC/NLA/P CNA 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 [Erratum in *J Am Coll Cardiol* 2019 Jun 25;73(24): 3234-7]. *J Am Coll Cardiol* 2019;73(24):3168-209. (PMID: 30423391)
16. Lloyd-Jones DM, Morris PB, Ballantyne CM, et al. 2017 Focused Update of the 2016 ACC Expert Consensus Decision Pathway on the Role of Non-Statin Therapies for LDL-Cholesterol Lowering in the Management of Atherosclerotic Cardiovascular Disease Risk: A Report of the American College of Cardiology Task Force on Expert Consensus Decision Pathways. *J Am Coll Cardiol* 2017;70(14):1785-822. (PMID: 28886926)
17. Bureau International des Poids et Mesures. JCGM 200:2012 International Vocabulary of Metrology-Basic and General Concepts and Associated Terms, 2008 Version with Minor Corrections. 3rd ed. https://www.bipm.org/documents/20126/2071204/JCGM_200_2012.pdf
18. Čelap I, Vukasović I, Juričić G, Šimundić AM. Minimum requirements for the estimation of measurement uncertainty: Recommendations of the joint Working group for uncertainty of measurement of the CSMBLM and CCMB [Erratum in *Biochem Med (Zagreb)* 2018 Feb 15;28(1):011201]. *Biochem Med (Zagreb)* 2017;27(3):030502. (PMID: 29180913)
19. Padoan A, Sciacovelli L, Aita A, Antonelli G, Plebani M. Measurement uncertainty in laboratory reports: A tool for improving the interpretation of test results. *Clin Biochem* 2018;57:41-7. (PMID: 29548924)
20. Plebani M, Sciacovelli L, Bernardi D, Aita A, Antonelli G, Padoan A. What information on measurement uncertainty should be communicated to clinicians, and how? *Clin Biochem* 2018;57: 18-22. (PMID: 29402416)
21. Milinković N, Ignjatović S, Šumarac Z, Majkić-Singh N. Uncertainty of Measurement in Laboratory Medicine. *J Med Biochem* 2018;37(3):279-88. (PMID: 30584397)
22. ISO 15189:2022. Medical laboratories - Requirements for quality and competence. Geneva: International Organization for Standardization (ISO), 2022. <https://www.iso.org/standard/76677.html>
23. Magnusson B, Näykki T, Hovind H, Krysell M, Sahlin E. Handbook for calculation of measurement uncertainty in environmental laboratories. Nordtest Report TR 537 (ed. 4) 2017. <https://www.nordtest.info/wp/2017/11/29/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-4/>
24. Aarsand AK, Fernandez-Calle P, Webster C, et al. The EFLM Biological Variation Database. <https://biologicalvariation.eu/> (Accessed at: 12.02.2025)

25. Tan R, Yilmaz M, Kurtulmuş Y. A practical approach example to measurement uncertainty: Evaluation of 26 immunoassay parameters. *Biochem Med (Zagreb)* 2022;32(3):030705. (PMID: 35966261)
26. Nurlu N, Cat A, Ucar KT. Measurement uncertainty in clinical chemistry: ISO 20914 versus nordtest or intermediate precision versus bias. *Scand J Clin Lab Invest* 2024 May;84(3):147-53. (PMID: 38742707)
27. Ustundağ Y, Huysal K. Measurement uncertainty of blood ethanol concentration in drink-driving cases in an emergency laboratory. *Biochem Med (Zagreb)* 2017;27(3):030708. (PMID: 29180916)
28. Kin Tekce B, Tekce H, Aktas G, Uyeturk U. The role of the uncertainty of measurement of serum creatinine concentrations in the diagnosis of acute kidney injury. *Ren Fail* 2016;38(2):305-10. (PMID: 26628060)
29. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs. the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA* 2013;310(19):2061-8. (PMID: 24240933)
30. Langlois MR, Chapman MJ, Cobbaert C, et al. Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and Very Low Concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM. *Clin Chem* 2018;64:1006-33. (PMID: 29760220)
31. Sampson M, Ling C, Sun Q, et al. A New Equation for Calculation of Low-Density Lipoprotein Cholesterol in Patients With Normolipidemia and/or Hypertriglyceridemia [Erratum in *JAMA Cardiol* 2020 May 1;5(5):613]. *JAMA Cardiol* 2020;5(5):540-8. (PMID: 32101259)
32. Langlois MR, Nordestgaard BG, Langsted A, et al. Quantifying atherogenic lipoproteins for lipid-lowering strategies: consensus-based recommendations from EAS and EFLM. *Clin Chem Lab Med* 2020;58:496-517. (PMID: 31855562)
33. Çubukçu HC, Topcu Dİ. Estimation of Low-Density Lipoprotein Cholesterol Concentration Using Machine Learning. *Lab Med* 2022 Mar 7;53(2):161-71. (PMID: 34635916)
34. Demirci F, Emec M, Gursoy Doruk O, et al. Prediction of LDL in hypertriglyceridemic subjects using an innovative ensemble machine learning technique. *Turk J Biochem* 2023;48(6):641-52. <https://www.degruyterbrill.com/document/doi/10.1515/tjb-2023-0154/html?srsId=AfmBOopFbwKvLpE3uDSdlm6K2OIGMqlik1LyuoYbqFFySf2ZB2BLhKW>