Calculated LDL-cholesterol: comparability of the extended Martin/Hopkins, Sampson/NIH, Friedewald and four other equations in South African patients

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Abstract

Aims: The reference method for low-density lipoprotein-cholesterol (LDL-C) is ultracentrifugation. However, this is unsuitable for routine use and therefore direct LDL-C assays and predictive equations are used. In this study, we compared the Friedewald, extended Martin/Hopkins, Sampson/NIH and four other equations to a direct assay.

Methods: We analysed 44 194 lipid profiles from a mixed South African population. The LDL-C predictive equations were compared with direct LDL-C assay and analysed using non-parametric statistics and error grid analysis.

Results: Both the extended Martin/Hopkins and Sampson/NIH equations displayed the best correlation with direct LDL-C in terms of desirable bias and total allowable error. The direct LDL-C assay classified 13.9% of patients in the low LDL-C (1.0-1.8 mmol/L) category, in comparison to the extended Martin/Hopkins equation (13.4%), the Sampson equation (14.6%) and the Friedewald equation (16.0%). The Sampson/NIH was least biased in the low LDL-C category (<1.8 mmol/L) and produced the least overall clinically relevant errors compared with the extended Martin/Hopkins and Friedewald equations in the low-LDL-C category.

Conclusions: Our findings suggest only a marginal difference between the extended Martin/Hopkins equation and the Sampson/NIH equation with the use of the Beckman Coulter DxC800 analyser in this population. The results favour the implementation of the Sampson/NIH equation when the Beckman Coulter DxC analyser is used, but the extended Martin/Hopkins may also be safely implemented. Both of these equations performed significantly better than the Friedewald equation. We recommend that patients be monitored using one of these methods and that each laboratory perform its own validation of either equation to ensure continuation and accuracy, and to prevent between-method variation.

Keywords: Hyperlipidemias; Lipids; Lipoproteins; Medical Laboratory Science; Quality control.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- The direct analyser used to measure the lipid profile may affect the performance of predictive low-density lipoprotein-cholesterol (LDL-C) equations. Previous studies evaluated the comparability of the predictive equations on Abbot and Roche analysers.

WHAT THIS STUDY ADDS

- The comparability of the extended Martin/Hopkins and Sampson/NIH equations was evaluated on the Beckman Coulter platform, yielding only a minimal overall difference between the two.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- As assay variability is likely to impact the performance of the predictive LDL-C equations, it is thus recommended that each chosen LDL-C method is validated in the local population cohort for each laboratory.

Introduction

The role of low-density lipoprotein-cholesterol (LDL-C) in risk assessment, diagnosis and management is well established, and recent clinical guidelines are still based on its use.[1, 2] The cost-effectiveness and well-known interpretation associated with the predictive equations count in favour of their continued use in comparison to the alternative biomarkers. The Friedewald equation, initially established as an alternative to the gold-standard ultracentrifugation, is still the most commonly used equation for LDL-C estimation in many clinical laboratories worldwide.[3, 4] However, in addition to its inaccuracy at LDL-C levels <1.8 mmol/L, it is formulated using the ratio of very LDL (VLDL)/triglyceride (TG) which loses its linearity in hypertriglyceridaemia. Current guidelines recommend that LDL-C be reduced to <1.8 mmol/L for secondary prevention in high-risk patients.[1, 5] With the availability of proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors on the market, these low LDL-C targets have become much more attainable.[1, 6] Subsequently, the need for accurate LDL-C estimation at lower LDL-C levels has become crucial in the clinical setting.

Direct LDL-C (dLDL-C) assays, such as the Friedewald equation, tend to lose accuracy in hypertriglyceridaemia and have variable performance between manufacturers as they are not standardised.[7–9] In addition, there are extra costs incurred in comparison to the use of equations. Miller *et al* evaluated several dLDL-C and high-density lipoprotein-cholesterol (HDL-C) methods and highlighted that for all LDL-C and HDL-C direct methods, the National Cholesterol Education Programme Ototal error goal of <12% was not met in diseased patients. The study also confirmed that in five of the eight methods evaluated, the Daiichi (now Sekisui medical) method included, samples with high TG (>1.6 mmol/L) were linked to increased LDL-C results in comparison to the reference method.[8]

Numerous modified predictive equations have thus been proposed over the last few years as alternatives to the Friedewald equation, aiming to improve on its shortcomings. The Martin/Hopkins and Sampson/NIH equations are particularly significant modifications. Martin *et al* [10] first described their equation in 2013, using an adjustable factor to calculate the ratio of

TG: VLDL-C. The equation has been widely reviewed and has demonstrated superiority over the Friedewald equation, especially at moderately high levels of TG and lower levels of LDL-C.[10, 11] The original Martin/Hopkins equation, such as the Friedewald equation, is not validated for TG levels >4.5 mmol/L (>400 mg/dL).[3, 10] A modification of the Martin/Hopkins equation uses an additional 240 cell stratification table (a total of 420 cells)[12] as opposed to the original 180 allowing use when TG levels are 4.5–9.0 mmol/L. The FOURIER trial and the 2018 US-Multi-society Cholesterol Guidelines recommended the Martin/Hopkins equation for calculating LDL-C when levels are <1.8 mmol/L, for example, patients treated with PCSK9 inhibitors.[2, 13, 14] These guidelines were, however, released prior to the description of the Sampson/NIH equation which was also proposed as a more accurate and practical LDL-C equation in patients with low LDL-C levels and high TG levels.[15] The Sampson/NIH equation first calculates the VLDL-C fraction providing a better estimate of the TG content in VLDL-C.[7, 15] An advantage of the Sampson/NIH equation is that it is more easily implementable on existing laboratory information systems when compared with the original or the extended Martin/Hopkins equations. A recent study performed by Sampson *et al* reports superior performance of the Sampson/NIH equation over the extended Martin/Hopkins and Friedewald equations at low LDL-C levels as well as a superior performance at high TG levels.[4] Both the Martin/Hopkins equation and the Sampson/NIH equations have been recommended by the 2021 ESC guidelines as alternatives (along with dLDL-C measurement) in patients where LDL-C is less than 1.8 mmol/L or high TG (<9.04 mmol/L) are present.[1] The guidelines do not, however, mention the clear advantages of these equations over the Friedewald equation.

Rossouw *et al* [16] evaluated 11 proposed LDL-C equations on two unpaired South African outpatient populations and concluded that the Martin/Hopkins equation may be safely implemented as an alternative to the less accurate Friedewald equation and that the Sampson equation is more likely to be affected by analyser choice. Steyn *et al* [17] evaluated four recent equations (Martin/Hopkins,[10, 12] Sampson/NIH,[15] Hattori,[18] Anandaraja[19]) on two separate South African populations: adult patients with diabetes and a paediatric cohort. The 2022 study by Steyn *et al* concluded that the Martin/Hopkins equation may be safely implemented as an alternative in adult diabetes and the Sampson/NIH equation for use in paediatric screening. It also further demonstrated the influence of analyser and reagent choice on the performance of the predictive equations.[17] Both studies comparatively evaluated the performance of these equations on the Abbott and Roche platforms. Martins *et al* [20] evaluated four LDL-C equations proposed at the time (Friedewald, Hattori, Chen, de Cordova), on a South African cohort of hospitalised patients and compared them to dLDL-C measurements done on the Beckman Coulter analyser. The findings suggested that the Hattori equation was the most suitable alternative to dLDL-C measurements in an adult, inpatient population in South Africa. This study, however, was published before the Martin/Hopkins and Sampson/NIH equations were formulated.

The National Health Laboratory Service (NHLS) laboratories that service the public sector in South Africa mostly still use the Friedewald equation to estimate LDL-C, with dLDL-C analysis reserved for samples with TG>4.5 mmol/L.

Our study sought to evaluate and compare the performance of the recently proposed LDL-C equations[10, 12, 15] and the Friedewald equation with dLDL-C measurement on the Beckman Coulter DxC analyser, on a combination of in- and outpatients of all ages and genders in the South African setting as a follow-up to the 2015 Martins *et al* study.

Materials and methods

Study population

This retrospective observational study used mined data of 44 194 lipid profiles (consisting of total cholesterol, TC; TG; HDL-C and dLDL-C), collected from patients in both inpatient and outpatient settings in Gauteng, South Africa between 1 January 2013 and 30 June 2013. All samples were analysed as part of routine clinical practice in real time and the data was thus retrospectively mined. The dataset included all ages as well as both sexes.

Laboratory tests

All components of the lipid profiles, including dLDL-C, were analysed concurrently on the Beckman Coulter DxC800 automated analyser (Brea, CA, USA) using Beckman Coulter reagents in an ISO15189-accredited NHLS Laboratory.

DLDL-C was measured using a homogeneous assay based on the Daiichi two-phase method that does not require any pretreatment or ultracentrifugation steps. Total cholesterol was measured using a colorimetric, enzymatic, time-endpoint method. TG measurements were carried out using a sequence of three coupled enzymatic steps to form a red quinone imine dye and the method used for HDL-C measurement was a homogeneous, colorimetric, enzymatic assay. The performance standards for these components were within acceptable coefficient of variation limits for Beckman DxC800, and the methodology has not changed over the last few years. All methods were part of standardised quality assurance/proficiency testing programmes.

The equations evaluated are listed in table 1.

Table 1

Predictive LDL-C equations evaluated in this study

	Equation	Units
Friedewald	$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5}$ $\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{2.2}$	mg/dL mmol/L
Martin/Hopkins	$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{f}$	mg/dL
Sampson	$\text{LDL-C} = \frac{\text{TC}}{0.948} - \frac{\text{HDL-C}}{0.971} - \left(\frac{\text{TG}}{8.59} + \frac{\text{Non-HDL-C}}{2140} - \frac{\text{TG}^2}{16100}\right) - 9.44$ $\text{LDL-C} = \frac{\text{TC}}{0.948} - \frac{\text{HDL-C}}{0.971} - \left(\frac{\text{TG}}{3.74} + \frac{\text{Non-HDL-C}}{24.16} - \frac{\text{TG}^2}{79.36}\right) - 0.244$	mg/dL mmol/L
Vujovic	$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{6.85}$	mg/dL
Puavilai	$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{6}$	mg/dL
Delong	$\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG} \times 0.16)$	mg/dL
Anandaraja	$\text{LDL-C} = 0.9\,\text{TC} - \left(\frac{0.9\text{TG}}{5}\right) - 28$	mg/dL

• HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.

Statistical analysis

Statistical analysis was performed using Microsoft Excel for Mac V.16.67 (Microsoft, Redmond, USA) and non-parametric data analysis. Bland-Altman difference plots were used to compare the predicted and directly measured LDL-C results. The differences were compared against a desirable bias of ±6.8% and a total allowable error (TE_a) of ±13.7% to assess the acceptability of between-method differences, as calculated by the biological variation estimates listed in the European Federation of Clinical Chemistry and Laboratory Medicine database.[23] The EFLM database contains biological variability information gathered from multiple studies, but unfortunately, no studies involving large biological variation databases specifically on African populations are available.[17]

Box and Whisker plots were used to compare the five-number summary between the datasets for each predicated equation. Clarke's error grid analysis (EGA) [4, 24] was performed on the entire cohort to evaluate the likelihood of clinically significant error for each LDL-C equation at low and high LDL-C cutoffs as defined by the 2018 US-Multi-society guidelines.[2]

Results

Characteristics of the study population

The dataset contained 44 194 lipid profiles that were analysed. 24 484 (55.4%) of the cohort were females, 19 532 (44.2%) were males and 178 (0.4%) were unspecified. A total of 2082 (5%) of the 44 194 lipid profiles in the dataset belonged to the paediatric population[17, 25–27] (0–18 years), consisting of 994 (47.7%) female patients, 1012 (48.6%) male patients and 76 (3.7%) unspecified patients. The median (percentiles) age was 54 (41–64) years. No patient-specific data in terms of clinical history, treatment or ethnicity were available. The dLDL-C data ranged from 0.17 to 29.17 mmol/L, mean 2.85 mmol/L±1.17 SD; HDL from 0.05 to 10.79 mmol/L, mean 1.20 mmol/L±0.40 SD; TC from 0.13 to 38.30 mmol/L, mean 4.73 mmol/L±1.46 SD and TG from 0.10 to 80.41 mmol/L, mean 1.76 mmol/L±1.49 SD.

The predicted and dLDL-C results are summarised in table 2 and figure 1.

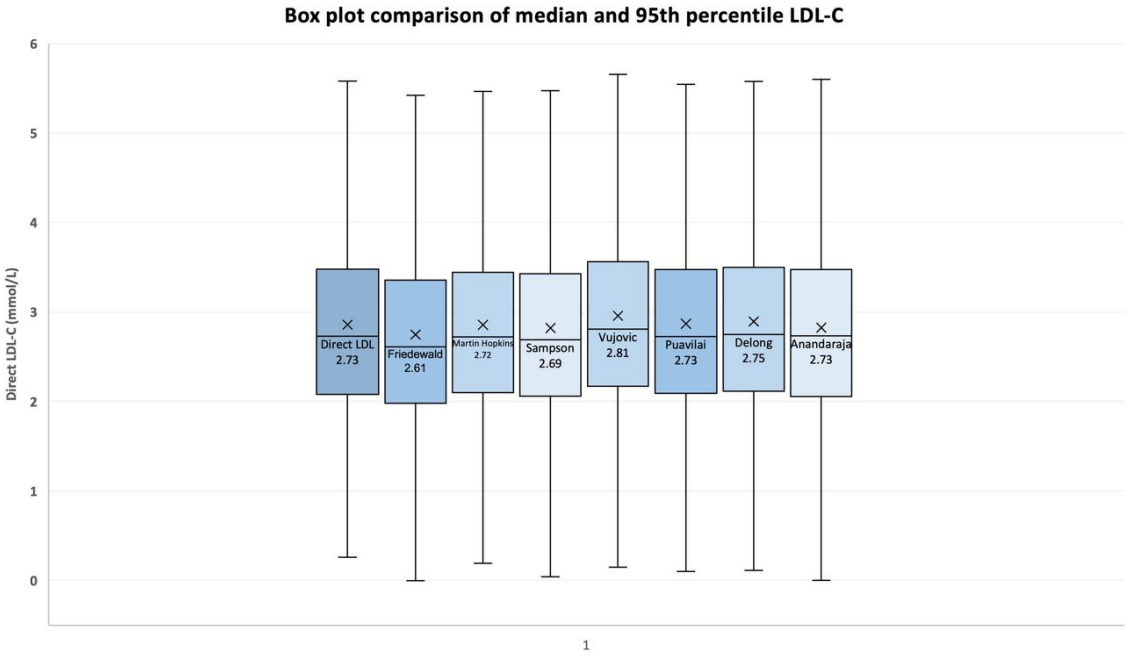

Figure 1

Box plot comparison of the predictive equations evaluated. LDL-C, low-density lipoprotein-cholesterol.

Table 2

Median low-density lipoprotein cholesterol (LDL-C) summary with percentage (%) calculated results that met error specifications.

	Median LDL-C (mmol/L)	IQR	P value	% of results within±6.8% (desirable bias)	% of results within ±13.7% (TEa)
Friedewald	2.61	(1.98–3.36)	<0.0001	50	80
Martin/Hopkins	2.72	(2.10–3.44)	<0.0001	59	89
Sampson	2.69	(2.06–3.43)	<0.0001	56	87
Vujovic	2.81	(2.17–3.56)	<0.0001	56	85
Puavilai	2.73	(2.09–3.47)	<0.0001	57	85
Delong	2.75	(2.11–3.50)	<0.0001	57	86
Anandaraja	2.73	(2.06–3.47)	<0.0001	34	62
Direct LDL	2.73	(2.07–3.48)	–	–	–

- **TEa, Total allowable error.**

Comparability of predictive equations versus dLDL-C

The Friedewald equation showed a negative proportional bias with a median bias of −3.6%, indicating the tendency to underestimate LDL-C values. Despite this, the Friedewald equation had a good correlation with the Beckman Coulter dLDL-C assay (r_s 0.945; $p<0.0001$).

The Martin/Hopkins equation showed a negative proportional bias with a median bias of −1.1%. The Martin/Hopkins equation showed similar performance to the Vujovic equation for having the best correlation of all the evaluated equations with the Beckman Coulter dLDL-C (r_s 0.957; $p<0.0001$).

The Sampson/NIH equation showed a negative proportional bias with a median bias of −1.6%. The Sampson/NIH equations had the second-best correlation to the Beckman Coulter dLDL-C of all the tested equations (r_s 0.956; $p<0.0001$).

The Vujovic, Puavilai, Delong, Anandaraja equations were all proportionally negatively biased. The Anandaraja equation had the worst correlation of all the evaluated equations (r_s 0.882; $p<0.0001$). The overall performances of these four equations were not superior to that of Martin/Hopkins and Sampson/NIH equations.

These results are summarised in table 2. The differences between the equations and the dLDL-C measurements are illustrated with Bland Altman plots in figure 2.

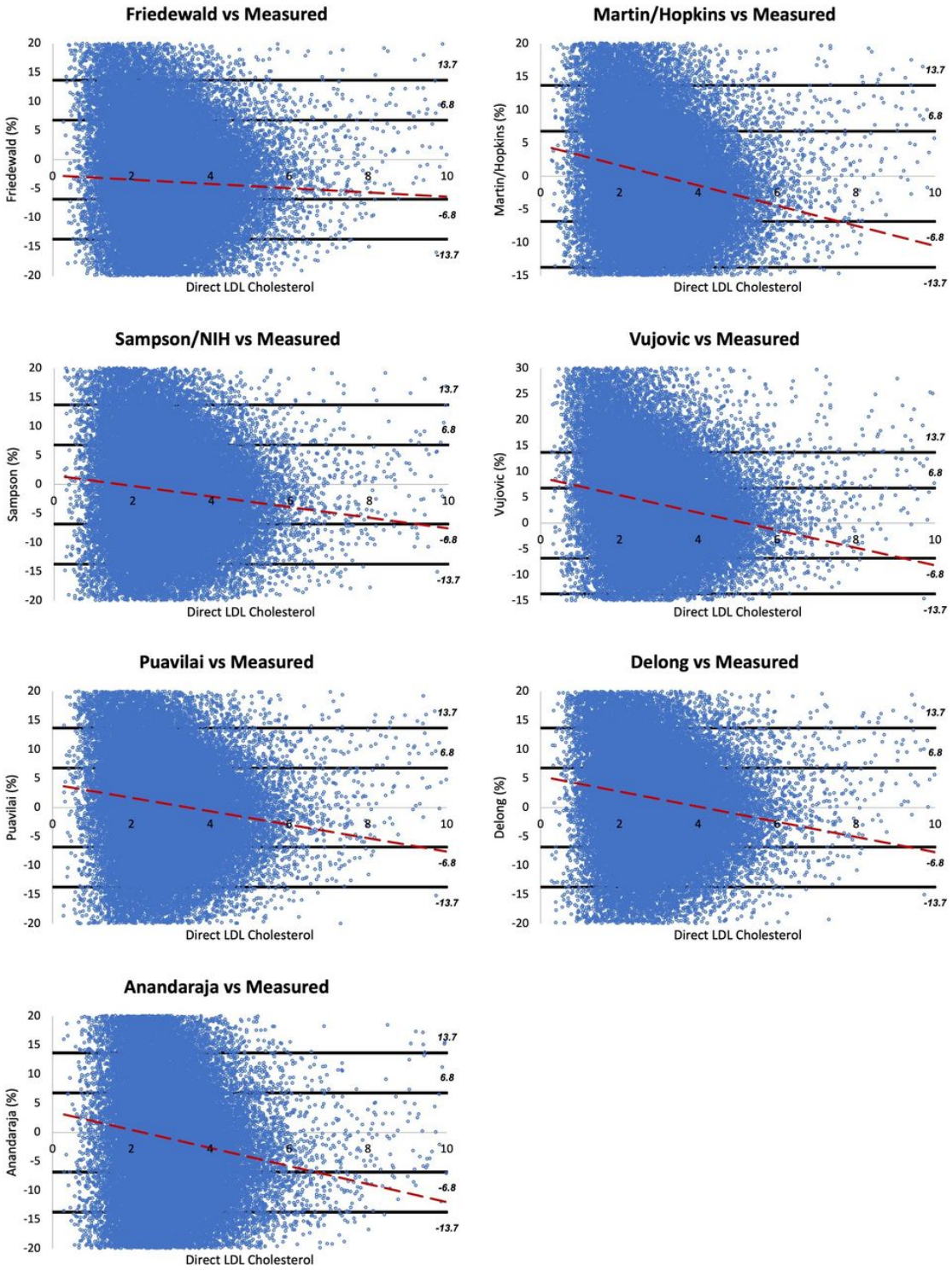

Figure 2

Difference (Bland-Altman) plots of the percentage bias between predictive LDL-C equations and direct LDL-C measurement. The solid black lines demonstrate the desirable bias (±6.8%) and the total allowable error (13.7%). LDL-C, low-density lipoprotein-cholesterol; NIH, National Institutes of Health.

The change in median bias relative to dLDL-C was investigated across LDL-C ranges and the results are summarised in table 3. The Sampson (0.8%) equation was least biased at LDL-C levels between 1.0 and 1.81 mmol/L and Martin/Hopkins followed with a median bias of 3.3%. At LDL-C values <1.0 mmol/L, both equations underestimated the LDL-C level, with the Martin/Hopkins equation yielding a larger bias (12.8%) than Sampson/NIH (7.5%). All seven equations maintained acceptable median biases (≤±6.8%) for LDL-C values >1.8 mmol/L.

Table 3

Median percentage (%) bias according to low-density lipoprotein cholesterol (LDL-C) level in mmol/L

	% bias according to LDL in mmol/L					
	< 1.00	**1.00–1.80**	**1.81–2.50**	**2.51–3.00**	**3.01–4.90**	**> 4.90**
Friedewald	**5.90**	**−1.19**	**−3.34**	**−4.12**	**−4.27**	**−3.16**
Martin/Hopkins	12.83	**3.26**	**0.08**	**−1.49**	**−2.57**	**−2.44**
Sampson	7.48	**0.75**	**−0.89**	**−1.83**	**−2.46**	**−2.92**
Vujovic	22.34	8.13	**4.08**	**2.21**	**1.14**	**1.53**
Puavilai	15.38	**4.08**	**0.90**	**−0.53**	**−1.19**	**−0.46**
Delong	17.12	**5.21**	**1.79**	**0.27**	**−0.53**	**0.08**
Anandaraja	−8.60	**0.19**	**−0.64**	**−2.19**	**−3.92**	**−5.06**

- **Results that met the desirable bias specification (±6.8%) are indicated in bold. The total allowable error specification is ±13.7%.**

Comparability across different TG levels

This study investigated the change in median bias relative to dLDL-C across the TG range on the Beckman Coulter analyser (table 4). The Friedewald equation exceeded the ±6.8% desirable bias specification when TG levels exceeded 1.7 mmol/L, and exceeded the TE_a (±13.7%) for TG levels >4.5 mmol/L.

COLLAPSE INLINE VIEW POPUP

Table 4

Median percentage (%) bias according to triglycerides in mmol/L

	% bias according to triglycerides in mmol/L			
	< 0.56	**0.56–1.69**	**1.7–4.5**	**4.5–9.0**
Friedewald	**5.00**	**−1.86**	−8.16	−21.18
Martin/Hopkins	**0.39**	**−1.82**	**−0.28**	7.31
Sampson	**2.96**	**−0.67**	**−3.91**	−9.81
Vujovic	7.81	**3.24**	**1.46**	**0.67**
Puavilai	**6.59**	**1.07**	**−2.65**	−8.68
Delong	6.95	**1.68**	**−1.52**	**−6.23**
Anandaraja	17.26	**1.20**	−9.43	−24.58

- **Results that met the desirable bias specification (±6.8%) are indicated in bold. The total allowable error specification is ±13.7%.**

In the defined 4.5 mmol/L – 9.0 mmol/L subgroup, which represented 3.4% (N=1482) of the study population, the extended Martin/Hopkins equation showed a positive median bias of 7.3% across all LDL-C levels. Friedewald (−21.2%) and Sampson/NIH (−9.8%) showed larger negative median biases as compared with the dLDL-C assay. The Vujovic (0.7%) and Delong (6.2%) equations were the only two equations that met the desirable bias specification in this subgroup.

Patients with TG levels between 1.7 and 4.5 mmol/L across all LDL-C levels represented 33.8% (N=14 951) of the study population. In this subgroup, the Martin/Hopkins equation (−0.3%) was the least biased when compared with dLDL-C. The Sampson/NIH equation (−3.9%) compared well too, meeting desirable and total error bias specifications.

These results illustrate the variability of the equations across TG levels. The differences between the Martin/Hopkins, Friedewald and Sampson/NIH equations against TG levels are illustrated in figure 3.

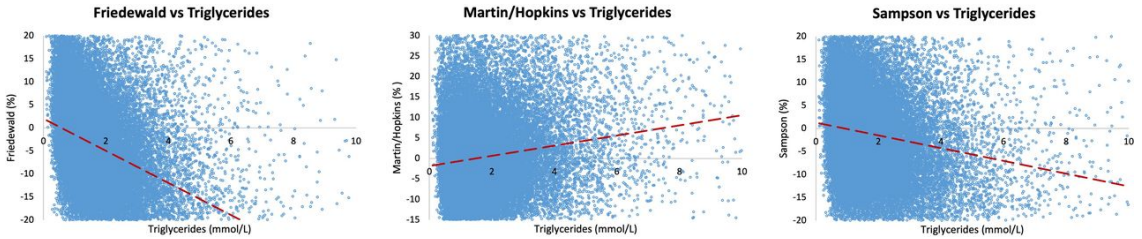

Figure 3

Difference (Bland-Altman) plots of the percentage bias between predictive LDL-C equations and triglyceride levels. LDL-C, low-density lipoprotein-cholesterol.

Patient classification in different LDL-C categories

The Martin/Hopkins and Sampson equations both performed well in comparison to the dLDL-C across all LDL-C ranges. The reclassification rate for the dLDL-C assay compared with the Martin/Hopkins equation was −0.1% to 1.1% (130–470 patients) across the entire range of LDL-C. In the low LDL-C (1.0–1.8 mmol/L) category, the dLDL-C assay classified 13.9% of patients in comparison to the Martin/Hopkins equation (13.4%), the Sampson equation (14.6%) and the Friedewald equation (16.0%). In the >4.9 mmol/L category, the dLDL-C assay classified 4.3% (1886) patients in comparison to Martin/Hopkins (1752) and Sampson (1739), which both classified 4.0%. The Friedewald equation classified 3.8% (1660) in the >4.9 mmol/L category. This illustrates the unacceptable misclassification rates of the Friedewald equation, overestimating results in the low LDL-C category (<1.8 mmol/L) and underestimating results in the high LDL-C category (>4.9 mmol/L). The classification according to equations is summarised in table 5 .

Table 5

Patient classification according to low-density lipoprotein cholesterol (LDL).

LDL-C levels	<1.00 mmol/L	1.00 - 1.80 mmol/L	1.81 - 2.50 mmol/L	2.51 - 3.00 mmol/L	3.01 mmol/L - 4.90 mmol/L	>4.90 mmo/L
	1037	**6097**	**10 866**	**8457**	**15 619**	**1886**
Direct LDL	2.4%	13.9%	24.7%	19.2%	35.5%	4.3%
Friedewald	**1376**	**7033**	**11 722**	**8136**	**14 035**	**1660**
	3.1%	16.0%	26.7%	18.5%	31.9%	3.8%
Martin/Hopkins	**816**	**5869**	**11 338**	**8750**	**15 437**	**1752**
	1.9%	13.4%	25.8%	19.9%	35.1%	4.0%
Sampson	**961**	**6437**	**11 348**	**8384**	**15 093**	**1739**
	2.2%	14.6%	25.8%	19.1%	34.3%	4.0%
Vujovic	**629**	**5407**	**10 604**	**8547**	**16 526**	**2249**
	1.4%	12.3%	24.1%	19.4%	37.6%	5.1%
Puavilai	**879**	**6119**	**11 138**	**8385**	**15 474**	**1967**
	2.0%	13.9%	25.3%	19.1%	35.2%	4.5%
Delong	**808**	**5916**	**11 011**	**8429**	**15 750**	**2048**
	1.8%	13.5%	25.0%	19.2%	35.8%	4.7%
Anandaraja	**1662**	**6077**	**10 445**	**8194**	**15 833**	**1751**
	3.8%	13.8%	23.8%	18.6%	36.0%	4.0%

- **Bold values show the numbers of individuals.**

Error grid analysis

We used EGA to comparatively evaluate analytical error for the Friedewald, extended Martin/Hopkins and Sampson/NIH equations (figure 4), and subsequently assess the likelihood of clinically significant error per equation. The TE_a goal of 13.7% was used, and the differences that exceeded 13.7% but did not result in incorrect classification were categorised as pure analytical errors. Differences that exceeded the TE_a goal and either overestimated or underestimated patients at the low (1.8 mmol/L) and high (4.9 mmol/L) LDL-C cut-offs1 2 were categorised as clinically relevant errors.

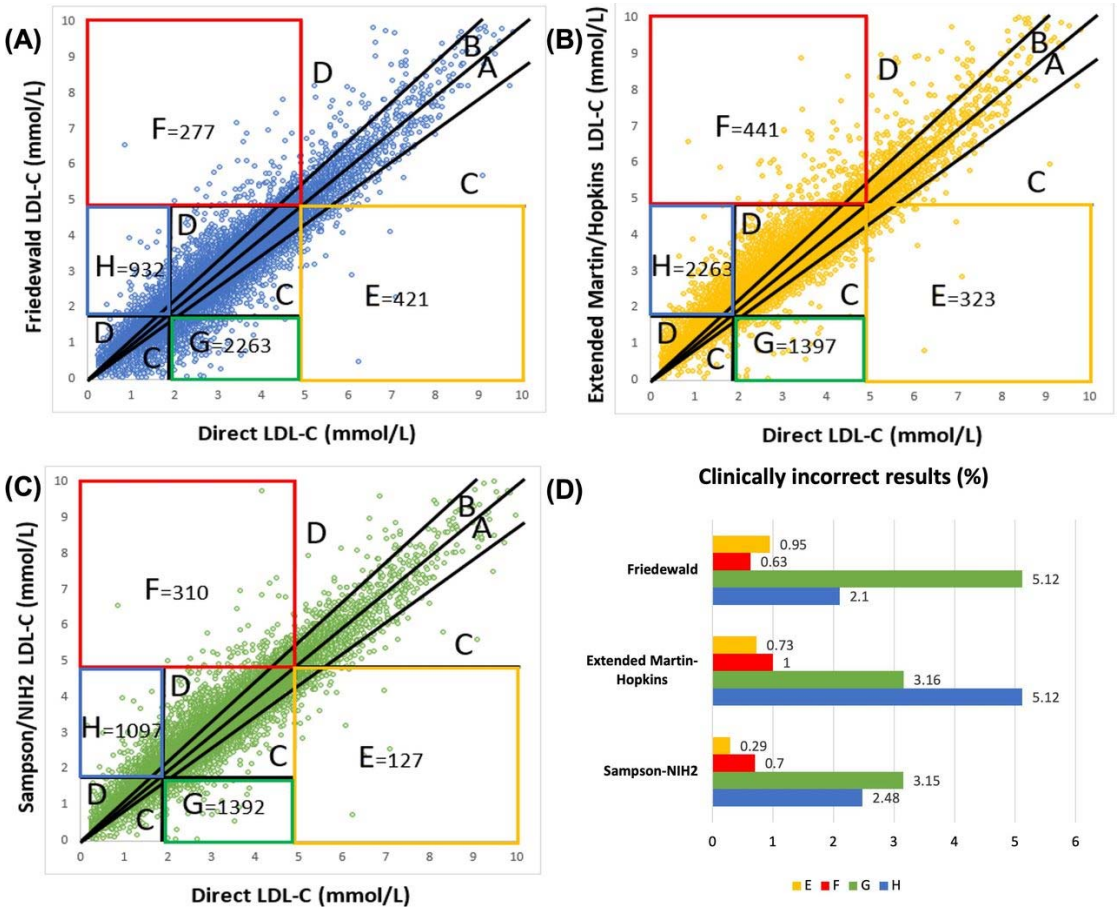

Figure 4

(A–C) Error grid analysis for predictive LDL-C by the Friedewald (A) extended Martin/Hopkins (B) and Sampson/NIH (C) equations in the whole cohort (N=44 194). Descriptions for errors are shown on graphs. (A) Within 13.7% proportional error and below the regression line, (B) within 13.7% proportional error and above the regression line, (C) greater than 13.7% proportional error but no impact on patient management and below the regression line, (D) greater than 13.7% proportional error but no impact in patient management and above the regression line, (E) underestimation of LDL-C at high LDL-C cut-point (4.9 mmol/L) leading to error in patient management, (F) overestimation of LDL-C at high LDL-C cut-point leading to error in patient management, (G) Underestimation of LDL-C at low LDL-C cut-point (1.8 mmol/L) leading to error in patient management, (H) overestimation of LDL-C at low LDL-C cut-point leading to error in patient management. Numbers in the highlighted zones (E–H) indicate the number of clinically relevant errors, (D) percentage of clinically relevant errors at the low (1.8 mmol/L) and high (4.9 mmol/L) LDL-C cut-offs according to zones (E–H). LDL-C, low-density lipoprotein-cholesterol; NIH, National Institutes of Health.

In this cohort, the extended Martin/Hopkins equation (10.0%) had the highest total percentage of incorrect results, and thus the highest likelihood of clinically relevant error. Friedewald followed with 8.8%, and Sampson/NIH proved to be the least likely to result in a clinically relevant error with a total of 6.6% clinically incorrect results (figure 4). The largest percentage of discrepant results leading to incorrect clinical classification was at the low LDL-C cut-off, where the Friedewald equation (5.1%) and Sampson/NIH (3.15%) equation were found to be more likely to underestimate LDL-C. In contrast, the extended Martin/Hopkins equation (5.1%) was likely to overestimate LDL-C leading to clinically relevant errors (figure 4) at the low LDL-C cut-off. At the high LDL-C cut-offs, the Sampson/NIH (0.7%) and extended Martin/Hopkins (1%) equations tended to overestimate LDL-C leading to clinically relevant error, whereas the Friedewald equation underestimated. The total clinically relevant errors of the extended Martin/Hopkins equations were compared with the Sampson/NIH equation, yielding a p value of 0.255 thus proving the difference statistically insignificant. While it is ideal to ensure maximum accuracy and avoid overestimation or underestimation of LDL-C results, in cases where a small percentage of error is unavoidable it seems preferable to overestimate patient results and still have them receive treatment as opposed to underestimating. The benefits of overestimating and treating outweigh the risks of underestimating and missing required treatment targets.

Discussion

The extended Martin/Hopkins equation has only been evaluated in a few recent studies[4, 17, 28] and not nearly as extensively as the original 180 cell variation. In this study, the extended Martin/Hopkins and Sampson/NIH equations showed the best overall correlation with the dLDL-C measurements in terms of allowable bias and TE_a. The Vujovic, Puavillai and Delong equations also performed well. The Friedewald and Anandaraja equations were the least comparable to dLDL-C in this study population.

At LDL-C levels <1.8 mmol/L, the Sampson equation was shown to be least biased (0.8%), with the Martin/Hopkins following with a positive bias of 3.6%. There is ongoing research and contrasting opinion regarding which equation is best suited to the low LDL-C subgroup. The 2018 US-Multi-society guidelines suggest the use of the Martin/Hopkins equation when LDL-C levels are <1.8 mmol/L,[2] while the 2021 Canadian Society of Clinical Chemists guidelines recommend the Sampson/NIH equation.[29]

A recent systematic review suggests that at TG levels>4 mmol/L, the Sampson/NIH equation was most accurate compared with the 180 cell Martin/Hopkins and Friedewald equations when compared with dLDL-C measurements.[30] The Vujovic and Delong equations were the only two to meet the desirable bias specification when TG>4.5 mmol/L in this study. Both the extended Martin/Hopkins and Sampson/NIH equations did not meet desirable bias specifications in this subgroup of the study, with the Sampson/NIH equation producing a greater bias than the extended Martin/Hopkins equation. In addition to the smaller bias, the Martin/Hopkins equation was the only one to overestimate LDL-C values compared with Sampson/NIH and Friedewald.

Clarke's EGA, which was initially used to assess the accuracy of self-monitoring glucometers,[24] was recently adapted for LDL-C equations by Sampson *et al* [4] and used to evaluate the likelihood of clinically relevant error. Sampson *et al* compared these equations to the gold-standard beta quantification method and found the Sampson/NIH equation to be least likely to produce analytically incorrect results. In our study, we evaluated the Sampson/NIH,

extended Martin/Hopkins and Friedewald equations with dLDL-C measurements and also found the Sampson/NIH equation to produce the least clinically relevant errors at high (4.9 mmol/L) and low (1.8 mmol/L) LDL-C cut-offs. The extended Martin/Hopkins equation had the highest total clinically relevant errors, with a greater tendency to overestimate LDL-C at both low and high cut-offs. However, the overall difference between total clinically relevant errors for the two equations was marginal and statistically insignificant (p=0.255). Furthermore, it is arguably better to overestimate residual cardiovascular risk than it is to underestimate, which could lead to exclusion from treatment as advocated by current guidelines.[1]

The study was limited by our lack of access to the reference method for LDL-C measurement. Furthermore, it has been reported that the reagent and automated analyser choice for dLDL-C measurement may influence the performance of the equations.[16, 17] In this study, results from a single platform were evaluated.

All lipid profile data were deidentified prior to analysis, therefore, it is unclear whether certain profiles were repeats for a single patient. We also did not have any clinical information specifying the use of lipid-modifying agents in the patient population. The study did, however, include a large sample population of inpatients and outpatients of all races, genders and ages that is representative of daily practice in the South African setting.

In conclusion, our findings suggest only a marginal difference between the extended Martin/Hopkins equation and the Sampson/NIH equation with the use of the Beckman Coulter DxC800 analyser in this population. The results favour the implementation of the Sampson/NIH equation when the Beckman Coulter DxC analyser is used, but the extended Martin/Hopkins may also be safely implemented. It is, however, clear that both of these equations performed significantly better than the Friedewald equation. We recommend that patients be monitored using the same method to ensure continuation and accuracy and to prevent errors arising from between-method variation. DLDL-C assays are not standardised,[8] and the results also tend to vary according to the analyser and reagent choice.[16, 17, 31] This is important to consider if laboratories opt to implement one of the newer equations, as the assay variability may impact the performance of the equation, and subsequently affect the risk classification and treatment decisions made by clinicians. Therefore, each LDL-C method on a specific analyser should be validated in the local population cohort for each laboratory.

Data availability statement

Data are available on reasonable request.

Ethics statements

Patient consent for publication

Not applicable.

Ethics approval

This study was approved by the Faculty of Health Sciences Research Ethics Committee at the University of Pretoria, approval number: 624/2022.

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Contributors

AC: conceptualisation, analysis interpretation, resources, writing (initial manuscript, review and editing). MR: resources, writing (review and editing). NS: resources, writing (review and editing). JM: conceptualisation, writing (review and editing). TSP: conceptualisation, visualisation, project administration, supervision, writing (review and editing), guarantor.

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