Comparison of measured LDL cholesterol with calculated LDL-cholesterol using the Friedewald and Martin-Hopkins formulae in diabetic adults at Charlotte Maxeke Johannesburg Academic Hospital/NHLS Laboratory.

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Student number: 1937608

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Chemical Pathology.

Johannesburg, September 2022

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DECLARATION

I, Mogomotsi Portia Dintshi, student number 1937608, declare that this research report is my work. I have contributed adequately to the findings issued in this report. It is being submitted for the Master of Medicine in Chemical Pathology under the Department of Chemical Pathology at the University of the Witwatersrand. This report has not been submitted for examination towards a degree at any other University.

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1. Dr Siyabonga Khoza (BSc, MBChB, FC Path (SA) (Chem), Mmed (Chem))

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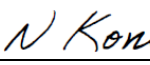
Signature:  Date: 06 January 2023

STATEMENT

The research report consists of a submissible article (including literature review), a study protocol, and appendices. Before undertaking this research project, a research protocol detailing the study's methodology was written, submitted for assessment, and approved by a panel of assessors from the Health Science Faculty at the University of the Witwatersrand School of Pathology. A copy of the research protocol for background reference and the ethics clearance certificate are included under the appendices of this research report.

The formatting of this report complies with the Health Sciences Faculty of the University of the Witwatersrand style outlined for Theses, Dissertations, and Research Reports. The format of the submissible article may differ from the rest of the research report to comply with the author's guidelines of the PLOS ONE Journal, to which it has been submitted.

DEDICATION

I dedicate this research report affectionately to the following:

My Partner

My Parents

My Brother and Sister

and

The Dintshi family

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors Drs S Khoza and N Kone for their immense support, knowledge, motivation and active role to ensure that this research was concluded in the best quality form and in record time.

Lastly, this research could not have been possible without the continuous encouragement and time provided by Professor Jaya George the chemical Pathology head of department at the University of the Witwatersrand.

ABSTRACT

Background

National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) and the European Society of Cardiology recommends using low-density lipoprotein cholesterol (LDL-C) as a treatment target for cholesterol lowering therapy. The Friedewald formula underestimate LDL-C in non-fasted and hypertriglyceridemia patients. This study aimed to compare measured LDL-C to calculated LDL-C in diabetic patients using the Friedewald and Martin-Hopkins formulae.

Methods

The data of 1 247 adult diabetes patients were retrospectively evaluated, and included triglycerides (TG), LDL-C, total cholesterol, and high-density lipoprotein cholesterol that were measured on the Roche Cobas® c702. Passing-Bablok regression analysis was used to determine the degree of agreement between measured LDL-C and calculated LDL-C using both formulae. The Bland-Altman plots were used to assess the bias at medical decision limits based on the 2021 European Society of Cardiology (ESC) guidelines on cardiovascular disease prevention in clinical practice.

Results

Both formulae showed a good linear relationship against measured LDL-C. However, the Martin-Hopkins formula outperformed the Friedewald formula at LDL-C treatment target <1.4mmol/L. The Friedewald formula and the Martin-Hopkins formula had 14.9% and 10.9% mean positive bias, respectively. At TG-C ≥1.7 mmol/L, the Martin-Hopkins formula had a lower mean positive bias of 4.2 % (95 % CI 3.0-5.5) compared to the Friedewald formula, which had a mean positive bias of 21.8 % (95 % CI 19.9-23), which was higher than the NCEP ATP III recommended total allowable limit of 12%.

Conclusion

The Martin-Hopkins formula performed better than the Friedewald formula at LDL-C of 1.4 mmol/L and showed the least positive bias in patients with hypertriglyceridemia.

Keywords: Friedewald; low density lipoprotein; Martin Hopkins

NOMENCLATURE

AARMS - Academic affairs and research management system

CVS - Cardiovascular system

CWD - Central data warehouse

CMJAH - Charlotte Maxeke Johannesburg academic hospital

CI - Confidence interval

EAS - European atherosclerosis society

EFLM - European federation of clinical chemistry and laboratory medicine

ESC - European society of cardiology

HDL - High-density lipoprotein

H_2O_2 - Hydrogen peroxide

LDL-C - Low-density lipoprotein cholesterol

NHLS - National health laboratory services

NCEP ATP III - National Cholesterol Education Programme Adult Treatment Panel III

OPD - Outpatient department

SD - Standard deviation

SCORE - Systemic coronary risk estimation

TC - Total cholesterol

TG – Triglycerides

VLDL - Very low-density lipoprotein.

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- **Figure 4**: Bland Altman Plot comparing measured LDL-C to the Friedewald formula and Martin Hopkins formula at TG-C of ≥ 1.7 mmol/L.

SUBMISSIBLE ARTICLE

Comparison of measured LDL cholesterol with calculated LDL-cholesterol using the Friedewald and Martin-Hopkins formulae in diabetic adults at Charlotte Maxeke Johannesburg Academic Hospital/NHLS Laboratory.

Mogomotsi Portia Dintshi, Ngalulawa Kone, Siyabonga Khoza

1. INTRODUCTION

Cardiovascular disease (CVD) accounts for nearly 40 million deaths per annum worldwide (1) of which diabetes mellitus is a major risk factor. According to the International Diabetes Federation, diabetes mellitus is estimated to affect 463 million adults worldwide and over 19 million adults in the African region in 2019 (2). The number of people living with diabetes mellitus is predicted to rise to 700 million adults by 2045, which represents an alarming 51% increase (2,3). Insulin resistance is a hallmark of diabetes mellitus, which is strongly linked to dyslipidemia (4). The lipid profile pattern associated with diabetes mellitus is elevated triglycerides (TG), low high-density lipoprotein (HDL), and elevated small dense low-density lipoprotein (LDL) (5,6). This is consistent with the findings of the Framingham study, which found that hypercholesterolemia is a risk factor for CVD and predicts CVD risk over a 10-year period (7).

LDL cholesterol (LDL-C) is recognized as a risk factor for CVD by the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) as well as the European Society of Cardiology (ESC) and it is used as a treatment target for cholesterol-lowering therapy (8,9). Patients are firstly categorized using the Framingham risk score (10) based on their risk factors and then each class of patients is assigned a target LDL-C

concentration to which lipid-lowering medications are targeted (8). This emphasizes the need to accurately report LDL-C levels to improve patient classification and management.

In the laboratory, LDL-C can be assessed using a variety of direct or indirect measurement methods. The gold standard method for LDL-C is the beta quantification by ultracentrifugation (11). However, this procedure is not ideal for routine clinical laboratory usage because it is cumbersome and time-consuming, and it also requires expert skills and a large sample volume (12). Various formulae for calculating LDL-C have been developed to solve these issues (13). Friedewald et al. originally proposed a formula to calculate LDL-C (14) and devised a method based on three parameters which include total cholesterol, triglycerides, and high-density lipoprotein (HDL). It was developed in 1972, from only 448 participants. However, this formula has limitations: it underestimates the LDL-C in non-fasted individuals with high chylomicron levels, and it is invalid at triglycerides (TG) of > 4.5 mmol/L (12) as it assumes a constant 5:1 ratio relationship between triglycerides and VLDL (15), even though the ratio has been proven to vary within and between individuals (16). Moreover, it relies on the accurate measurement of total cholesterol, HDL-C, and TG parameters used in the calculation. As a result, serum samples from non-fasted patients who have conditions associated with high TG levels such as uncontrolled diabetes mellitus, dysbetalipoproteinemia or alcoholism, may underestimate calculated LDL-C (17). This could lead to patients being misclassified for lipid-lowering treatment and predispose them to risk for cardiovascular complications.

Several studies have since revealed these flaws, and others have attempted to develop new formulae that better correlate with measured LDL-C testing. One such formula is the Martin-Hopkins formula which is recommended by both the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the European Atherosclerosis Society (EAS) because of the advantage it offers over the Friedewald formula, particularly at LDL-C concentrations of <1.8 mmol/L, TG concentration 2.0-4.5 mmol/L and, in non-fasting samples (18). Several studies have shown a good correlation between the Friedewald formula and the Martin-Hopkins formula in the general population (15,19), it is important that this formula is assessed in different populations before widely used. This study aims to compare measured LDL-C with calculated LDL-C using the Friedewald and

Martin-Hopkins formulae in diabetic patients over a range of LDL-C and TG concentrations.

2. MATERIALS AND METHODS

I. Study Design

We retrospectively reviewed the lipid profile data that was requested from Charlotte Maxeke Johannesburg Academic Hospital's (CMJAH) adult diabetic outpatient department (OPD) from August 2016 to December 2019. Before August 2016, LDL-C was determined using the Friedewald formula at CMJAH's National Health Laboratory Service (NHLS); but thereafter, an LDL homogeneous assay was adopted.

This study complied with the institutional regulations and was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance certificate No. M200858) and followed the Declaration of Helsinki. The data for the study was requested from the NHLS Central Data warehouse (CDW) after obtaining permission from Academic Affairs and Research Management System (AARMS), approval number PR20218. The data included triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) as well as high-density lipoprotein cholesterol (HDL-C).

A total of 1 301 participants who met the inclusion criteria were enrolled in the study. The following inclusion criteria were used: adult participants of ≥18 years old, both male and female participants from the diabetic OPD whose full lipid profile results were performed at CMJAH's NHLS. Participants who were non-diabetics, <18 years old, and with incomplete lipid profile were excluded.

II. Laboratory tests measurements

The Roche Cobas® c702 (Roche Diagnostics, Mannheim, Germany) was used to analyze lipid profiles according to manufacturer's instructions.

In summary, LDL-C was measured using a method that involves selectively solubilizing it with a surfactant which then combines with cholesterol esters and oxidase to yield Δ^4-cholestenone + hydrogen peroxide (H_2O_2). The H_2O_2 reacts with peroxidase, and

generates a red purple pigment (20). For TC measurement, the first step consists of cholesterol esters cleavage and oxidation, with the resulting products triggering oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye that is quantified in the presence of peroxidase.

The measurement of HDL-C requires that LDL-C, VLDL-C, and chylomicrons be excluded, by complexing them with dextran sulfate in the presence of magnesium ions. HDL-C esters are cleaved and oxidized into Δ^4-cholesterol and H_2O_2. The latter (H_2O_2), then reacts with Δ^4-amino-antipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline in the presence of peroxidase to form a purple-blue color. Lastly, in the TG method, the free glycerol is first blanked from the reaction before the hydrolysis of triglycerides by lipase. The liberated glycerol undergoes multiple subsequent enzyme catabolism to finally form a colored compound, 4-(p-benzoquinone-monoimino)-phenazone. The intensity of the color of various components of the lipid profile is directly proportional to the concentration of the analyte of interest.

We then estimated LDL-C from the given lipid profile results, using Friedewald and Martin-Hopkins formulae to assess the correlations between each formula and measured LDL-C, as well as between the two formulae.

The Friedewald formula used was as follows:

$$\text{LDL-cholesterol (mmol/L)} = Total\ cholesterol - HDL - \frac{Triglyceride}{2.2}$$

For Martin-Hopkins calculated LDL-C, the following formula was applied:

$$\text{LDL-cholesterol (mmol/L)} = Total\ cholsterol - \text{HDL} + \frac{Triglyceride}{adjustable\ factor}$$

The adjustable factor was obtained from 180-cell table strata using the participants' TG and non-HDL providing a more individualized approach. Non-HDL-C was obtained by subtracting HDL-C from total cholesterol. LDL-C in the Martin-Hopkins formula was calculated using a Microsoft Excel spreadsheet obtained from Johns Hopkins Medicine (21).

III. Statistical analysis

The data were entered and categorized using Microsoft Office Excel 2016 (Microsoft, Seattle, WA, USA). Statistical analyses were performed using MedCalc for Windows, version 19.8 (MedCalc Software, Ostend, Belgium). The Tukey test was performed for outlier detection. A total number of 54 results were excluded from the 1301 original sample size and the resulting study participants were 1247. The D'Agostino-Pearson test was used for normal distribution testing. Normally distributed data were expressed as mean and standard deviation. The LDL-C data was categorized based on the 2021 European Society of Cardiology (ESC) guidelines on cardiovascular disease prevention in clinical practice (9). The following LDL-C (mmol/L) treatment cut-offs were used; <1.40, <1.80, <2.60 and <3.00 (9). As there are presently no published therapeutic treatment goals for triglycerides, we classified TG levels as low and high risks if they were less and more than 1.7 mmol/L, respectively.

The method comparison between measured and calculated LDL-C formulae was performed according to Passing-Bablok regression analysis. As stated earlier, the reference method, beta-quantitation by ultracentrifugation, was not fit for use in our busy routine setting as it is time-consuming and expensive. Hence, we used the Bland- Altman plots to determine and demonstrate the degree of agreement as well as the level of bias between measured LDL-C and calculated LDL-C. For reason stated above, the beta quantitation was not used as a reference method for our analysis.

The total allowable limits of ±12% which is recommended by NCEP ATP III (8), was used to measure the agreement between measured LDL-C and calculated LDL-C. A p-value of <0.05 was considered statistically significant.

3. RESULTS

The study comprises a total of 1247 participants, including 57.2% females and 41.5% males. The study populations' mean age was 55.4 years with a standard deviation (SD) of 15.5. The characteristics of the study population are shown in Table 1.

Table 1: Demographic and biochemical characteristics expressed as mean ± SD.

LDL- C, low density lipoprotein cholesterol, HDL-C, high density lipoprotein cholesterol and SD, standard deviation

Variable	Mean ± SD
Age (years) • Males • Females	55.3 ±15.5 53.4 ± 15.2 57.2 ± 15.4
Total cholesterol (mmol/L) • Males • Females • Sex not stated (N =16)	4.07 ±0.95 2.42 ± 0.81 2.44 ± 0.80 2.30 ± 0.60
Triglyceride (mmol/L)	1.45 ±1.02
Non-HDL-C (mmol/L)	2.79 ±0.92
Measured LDL-C	2.44 ±0.80
LDL-C (Friedewald formula)	2.13 ±0.78
LDL-C (Martin-Hopkin Formula)	2.22 ±0.78

139139

In this study, the calculated LDL-C derived from the Friedewald formula was compared to the measured LDL-C. The regression analysis equation for Friedewald formula was y = -0.201 + 0.966x (r=0.952, 95% confidence interval (CI) 0.947 to 0.957, p-value <0.0001). (Fig 1A).

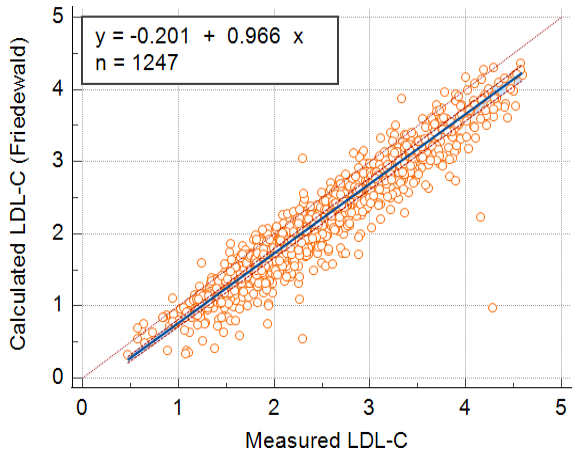
A good linear relationship was observed between the methods. We then compared the calculated LDL-C derived from the Martin-Hopkins formula to the measured LDL-C. The regression analysis equation for calculated LDL-C using Martin-Hopkins with measured LDL-C was y = -0.134 + 0.963 x (r= 0.954, 95% CI 0.949 to 0.959, p-value <0.0001), (Fig

1B). The Martin-Hopkins formula showed a better linear relationship with measured LDL-C when compared to the Friedewald formula.

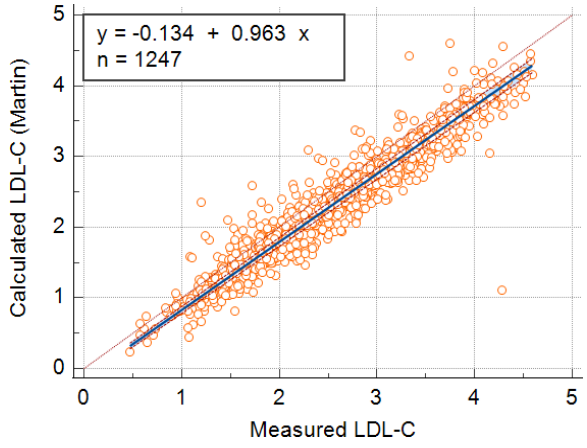
Finally, the Martin-Hopkins formula was compared to the Friedewald formula, we found that the regression analysis equation y = 0.0200 + 1.000 x had an even greater correlation. The correlation coefficient was 0.960, 95% CI 0.956 to 0.964 with a p-value p <0.0001, (Fig 1C).

Fig 1: The comparison of calculated to measured low-density lipoprotein (LDL-C) using Passing- Bablok regression analysis. (A) Friedewald formula and measured LDL-C, (B) Martin Hopkins formula and measured LDL-C and lastly (C) Friedewald formula and. Martin Hopkins formula**.**

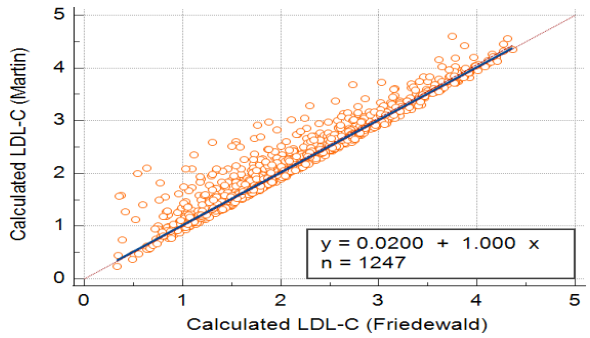
(A)



(B)



(C)



To improve the comparison between the measured and calculated LDL-C, the regression analysis equations were obtained at different LDL-C treatment targets (Table 2 and S1 Fig). The Martin-Hopkins formula performed better than the Friedewald formula at LDL-C of 1.4 mmol/L. There was a positive linear relationship between calculated and measured LDL-C methods. At LDL-C of 2.6 mmol/L and ≥3.0 mmol/L, we found a significant correlation coefficient with the Martin-Hopkins formula when compared to the Friedewald formula (Table 2).

Table 2: Summary of the comparison between measured and calculated low-density lipoprotein (LDL-C), using the Friedewald and Martin Hopkin formulae as well as regression analysis at different LDL-C levels.

LDL-C level	N number	Correlation coefficient (r^2)	Gradient (95% confidence interval)	Y intersection (95% confidence interval)	p-value
<1.40 mmol/L	95	Friedewald 0.650 Martin-Hopkins 0.682	1.29 (1.00 to 1.70) 1.43 (1.16 to 1.91)	-0.50 (-01.00 to 0.19) -0.65 (-1.25 to -0.33)	<0.0001 <0.0001
<1.80 mmol/L	187	Friedewald 0.576 Martin-Hopkins 0.499	2.33 (2.00 to 2.78) 2.44 (2.00 to 3.00)	-2.39 (-3.13 to -1.85) -2.49 (-3.37 to -1.77)	<0.0001 <0.0001
<2.60 mmol/L	482	Friedewald 0.710	1.30 (1.20 to 1.40)	-0.92 (-1.15 to -0.72)	<0.0001

		Martin-Hopkins 0.745	1.26 (1.17 to 1.35)	-0.77 (-0.96 to –0.58)	<0.0001
<3.00 mmol/L	191	Friedewald 0.539 Martin-Hopkins 0.521	2.29 (1.94 to 2.81) 2.20 (1.83 to 2.67)	-3.90 (-5.34 to –2.94) -3.58 (-4.90 to –2.56)	<0.0001 <0.0001
≥3.10 mmol/L	292	Friedewald 0.798 Martin-Hopkins 0.831	1.13 (1.06 to 1.22) 1.11 (1.04 to 1.19)	-0.85 (-1.15 to –0.57) -0.70 (-0.97 to –0.45)	<0.0001 <0.0001

172172

173 The Bland-Altman plots for Friedewald and Martin-Hopkin formulae showed a mean
174 positive bias of 14% (95% CI 14.1-15.6) and 10.24% (95% CI 9.59-10.90), respectively
175 (See fig 2A and 3B).

176 **Fig 2**: Bland Altman Plot comparing measured LDL-C and the Friedewald formula (A). There is a positive
177 mean bias of 14.9% (95% confidence interval 14.1 to 15.6) between the two methods. The total error
178 allowable (TEa) of 12% is indicated by the arrow. Bland Altman Plot comparing measured LDL-C and the
179 Martin Hopkins formula (B). There is a mean positive bias of 10.2% (95% confidence interval 9.6-10.9)
180 which is below the total error allowable of 12% (indicated by the arrow) as recommended by NCEP ATP III.
181 MH, Martin-Hopkins formula.

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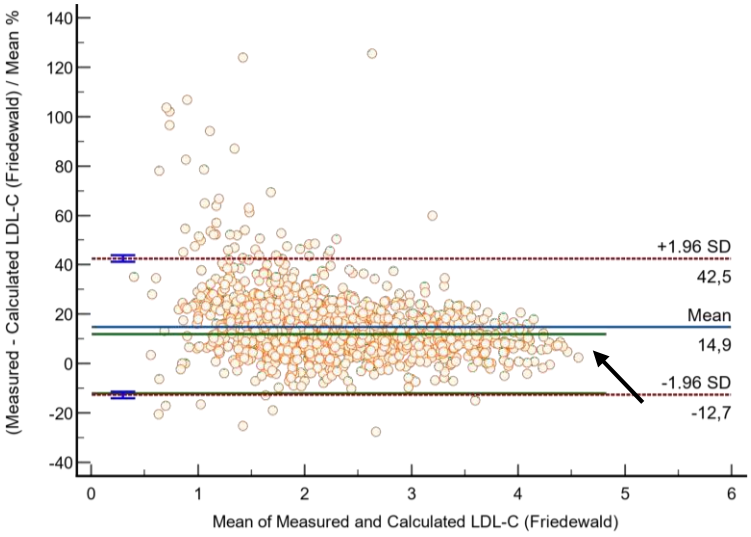
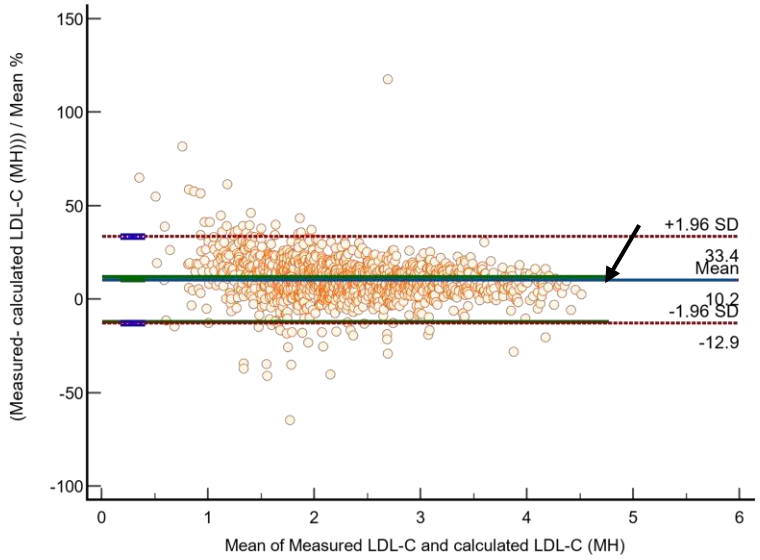
8
2

Fig 2A


(Measured - Calculated LDL-C (Friedewald) / Mean %
140
120
100
80
60
40
20
0
-20
-40
+1.96 SD
42,5
Mean
14,9
-1.96 SD
-12,7
0
1
2
3
4
5
6
Mean of Measured and Calculated LDL-C (Friedewald)

184 Fig 2B



185185

186 Significant correlations were found across the measured and calculated LDL-C at TG of
187 < 1.7 mmol/L and ≥ 1.7 mmol/L with Spearman correlation coefficient ranging from 0.962
188 to 0.949. There were 349 (28%) participants who had high-risk triglyceride concentrations
189 (≥ 1.7mmol/L). At TG-C of ≥1.7 mmol/L, the Friedewald formula showed a mean positive
190 bias of 21.8% (95% CI 19.9-23) which was higher than NCEP ATP III's recommended
191 total allowable limits of 12 %. At TG-C of ≥1.7 mmol/L, the Martin-Hopkins formula showed
192 a reduced mean positive bias of 4.2 % (95% CI 3.0-5.5) (Table 3 and Fig 3A and 4B).

193 **Table 3**: The mean bias, regression equation, correlation coefficient and p-value of measured and
194 calculated low-density lipoprotein at different triglycerides (TG) cut-offs.

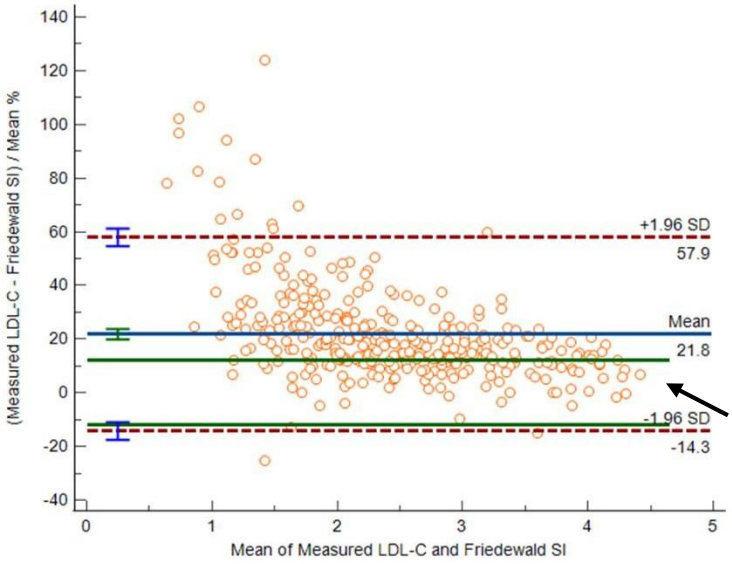
	TG <1.7 mmol/L (N=898)		TG ≥1.7 mmol/L (N=349)	
	Friedewald Formula	Martin-Hopkins Formula	Friedewald Formula	Martin-Hopkins Formula
Mean bias (95% Confidence interval)	12.3% (11.5-13)	12.6% (11.9-13.3)	21.8% (19.9-23.8)	4.2% (3.0-5.5)

Regression equation	y = - 0.161 + 0.965 x	y = - 0.148 + 0.957 x	y = - 0.448 + 1.012 x	y = 0.071 + 0.920 x
Correlation coefficient	0.963	0.962	0.949	0.949
p-Value	<0.0001	<0.0001	<0.0001	<0.0001

195195

196 **Fig 3**: Bland Altman Plot comparing measured LDL-C to the Friedewald formula and Martin Hopkins
197 formula at TG-C of ≥ 1.7 mmol/L. (A) There is a positive mean bias of 21.8% (95% confidence interval 19.9-
198 23) between measured LDL-C and Friedewald formula. and a reduced positive bias of 4.2% (95% CI 3.0-
199 5.5) with the (B) Martin-Hopkins formula. The total allowable limit of 12% is indicated by the arrow.

200 (A)



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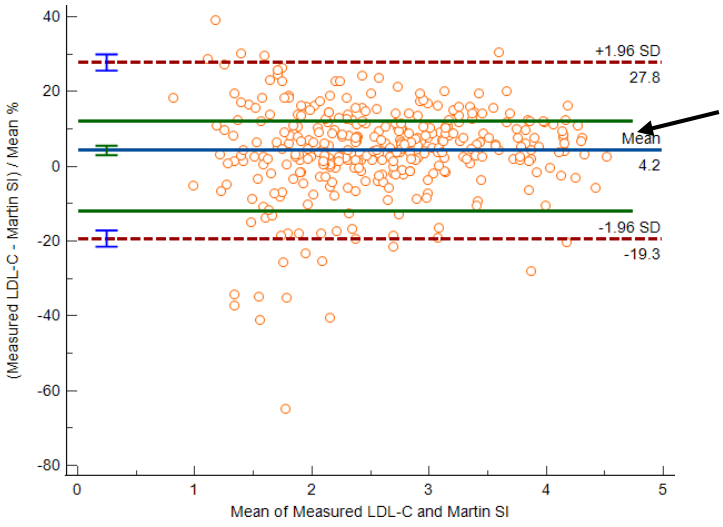
(B)



4. Discussion

We compared measured LDL-C to calculated LDL-C using the Friedewald and Martin-Hopkins equations in this study, which looked at the correlation of LDL-C methodologies in the South African diabetic community. By using linear regression analysis, we were able to demonstrate that both formulae had a good correlation. However, the Martin-Hopkins formula proved to have a better correlation, particularly at lower LDL-C concentrations of <1.4 mmol/L and had the least mean positive bias when compared to the Friedewald formula. The Friedewald formula significantly underestimates LDL-C in hypertriglyceridemia.

These results build on existing evidence by Rossouw et al. in a study also performed in the South African population. This latter study evaluated approximately 14000 out-patient lipid profiles comparing the performance of Friedewald, Martin-Hopkins and the Sampson formulae to measure LDL-C assays. They found that the Martin-Hopkins formula best estimated calculated LDL-C at low LDL-C of ≤1.8mmol/L, and at moderate hypertriglyceridemia 1.7-4.5 mmol/L compared to other formulae (22). In addition, a study by Martin et al. demonstrated that the Martin-Hopkins formula was more accurate than the Friedewald formula in low LDL-C concentrations (23). With the current debate of measuring fasted vs non-fasted LDL-C samples, Sathiakumar et al. evaluated the

accuracy of Martin-Hopkins and the Friedewald formulae in relation to fasting status (24). Their findings were that the Martin-Hopkins formula outperformed the Friedewald formula in both fasted and non-fasted samples. Furthermore, the Martin-Hopkins formula was found to be superior at low LDL-C concentration particularly in non-fasted samples.

It is agreed that accurate measurement of LDL-C is pivotal in ensuring correct assessment of patients' cardiovascular risk and treatment of dyslipidemia, targeted at lowering their LDL concentration (25). In 2021, ESC the published guidelines recommending that patients be first categorized according to the Systemic Coronary Risk Estimation 2 (SCORE2) and Systemic Coronary Risk Estimation 2-Older Persons (SCORE2-OP). The SCORE2 and SCORE2-OP estimates an individual's ten-year risk of fatal cardiovascular disease based on modifiable and non-modifiable risk factors. The categories include very-high, high, moderate, and low-risk (24). Patient's with well controlled diabetes mellitus of less than 10 years' duration and no evidence of target organ damage are categorized as moderate risk. The high-risk category represents patients with diabetes mellitus without any atherosclerotic cardiovascular disease and with or without target organ damage. The very-high risk category represents patients with high-risk features and in addition have with renal impairment or the presence of microvascular disease (24). Each category is assigned a specific LDL-C target concentration, emphasizing the importance of accurate and precise LDL-C methods to avoid misclassification and mismanagement of patients. The higher an individual's SCORE2 and SCORE2-OP, the lower the target LDL-C. The SCORE2/SCORE2-OP for the very-high risk category is ≥10%, with an LDL-C treatment target of 1.4 mmol/L. The very-high risk category includes people with documented atherosclerotic cardiovascular disease (acute coronary syndrome, stable angina, coronary revascularization, stroke and transient ischemic attack), diabetes mellitus with target organ damage, type 1 diabetes mellitus for more than 20 years' duration, severe chronic kidney disease or familial hyperlipidemia with atherosclerotic cardiovascular disease (26).

The Friedewald formula, which has long been used to calculate LDL-C levels, uses a fixed TG: cholesterol ratio as a proxy of VLDL-C, without considering chylomicrons which have higher TG than VLDL-C (12). This is unlikely to be true in clinical practice, since

patients are not always fasted. Therefore, the Friedewald formula is likely to overestimate VLDL-C and LDL-C in hypertriglyceridemia states and thus has been shown to be increasingly inaccurate at TG concentration between 2.3 to 4.5mmol/L (12). Multiple studies have demonstrated that this formula underestimates LDL-C, resulting in cardiovascular risk misclassification (27,28). As a result, numerous LDL-C formulae have been created, showing the benefits of being cost-effective, with a faster turnaround time, and simple compared to measured. There are, however, not without drawbacks. Several researchers have compared these LDL-C formulae in the past. Karkhaneh et al. (13) for example, compared eight equations in 2752 participants and found that the Friedewald formula overestimates LDL-C at TG values of 3.38mmol/L. A study by Sampson et al. also supported the evidence that the Martin-Hopkins formula was superior to the Friedewald formula in patients with low LDL-C as well as those with hypertriglyceridemia (24).

The Martin-Hopkins formula was first derived and validated in 2013 by Martin et al. in a study population of approximately 1.3 million fasted and nonfasted participants (16). The Martin-Hopkins formula replaced the fixed TG: VLDL ratio with an adjustable factor derived from a 180 cell strata table by dividing TG by non-HDL. Non-HDL is calculated by subtracting HDL from total cholesterol. Martin et al. found that the Martin-Hopkins formula had good correlation to directly measured LDL-C compared to the Friedewald formula and further studies demonstrated this finding as well (16). Our study has shown that the Martin-Hopkins formula is more accurate than Friedewald formula in diabetic patients. Therefore, it may be beneficial to apply the Martin-Hopkins formula when the measurement LDL-C concentration is not possible and inaccurate.

A similar conclusion was reached by Kang et al. when they evaluated four alternative LDL-C formulae in a Korean population and found that the Martin-Hopkins formula produced the least overestimation and underestimation of LDL-C values when compared to the Friedewald formula (15). However, Lee et al. discovered that the Martin-Hopkins formula overestimated LDL-C in the Korean population, implying a possible racial variance (19), and underlining the need for additional validation of such formula in other populations.

Additional evidence comes from a study by Ferrinho et al. which found that while both the Friedewald and Martin-Hopkins formulae showed good correlation with measured LDL-C, the latter performed better in samples with low LDL-C (2.6 mmol/L) and diabetic participants (29). Even though the Martin-Hopkins and Friedewald formulae all exhibited a good correlation to measured LDL-C, Song et al. found that the Martin-Hopkins formula was superior to the Friedewald formula across all TG values, especially in patients with dyslipidemias (30), the findings which are consistent with this study . These findings were confirmed by Sathiyakumar et al. who found that at TG concentrations of >4.5mmol/L, the Martin-Hopkins formula had a mean absolute deviation difference of 0.3, while the Friedewald formula had the lowest accuracy (31). Jagesh et al. also demonstrated that the Martin-Hopkins formula achieved a better precision at higher TG concentrations compared to the Friedewald formula (32).

Miller et al. first demonstrated that the Roche Diagnostic method compared to the gold standard method had an imprecision ranging from 1.3-1.9% and a mean bias of -3.9% which is within the recommended imprecision of ≤4% and mean bias of ≤4% by the NCEP ATP III as well an acceptable total error of 11% (33). Furthermore, Miller et al. in 2010 showed that the direct Roche Diagnostic method had a persistent negative bias when compared to the gold standard method and this finding was more profound in patients that were treated for CVD (34).

Strengths and limitations.

Our study had several strengths: (i) Our data was derived from those who are most likely to develop dyslipidemia in a real-world environment and, (ii) it compared measured LDL-C to Friedewald and Martin-Hopkins across a wide range of TG concentrations. Yet, it may have shown some limitations due to the lack of comparison between the Roche assays and the reference method. The used Roche assay, which has its own bias compared to the reference method, cannot be assumed to represent “true LDL-C”. In addition, homogeneous assays for measuring LDL-C are routinely used and recommended by the NCEP (35) and their performance is within specifications by NCEP ATP III (32).

Conclusion

We conclude that both the Martin-Hopkins and Friedewald equations have a strong correlation to measured LDL-C levels in the general population. The Martin-Hopkins formula outperformed the Friedewald formula in the South African diabetic population across all LDL concentrations, particularly at low concentrations of 1.4mmol/L and at hypertriglyceridemia of 1.7mmol/L. We demonstrated that the Martin-Hopkins formula generates more accurate findings than the Friedewald formula.

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None declared.

AUTHOR'S CONTRIBUTIONS

All authors have accepted responsibility for the entire content of this manuscript and approve its submission.

COMPETING INTERESTS

The authors state no conflict of interest.

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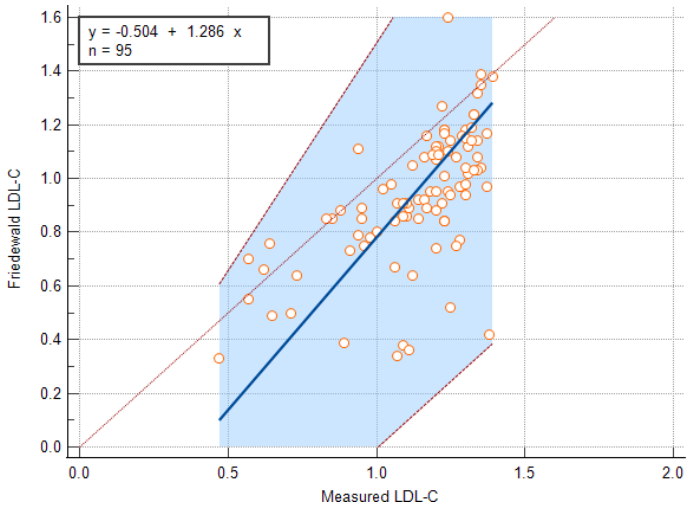
APPENDICES.

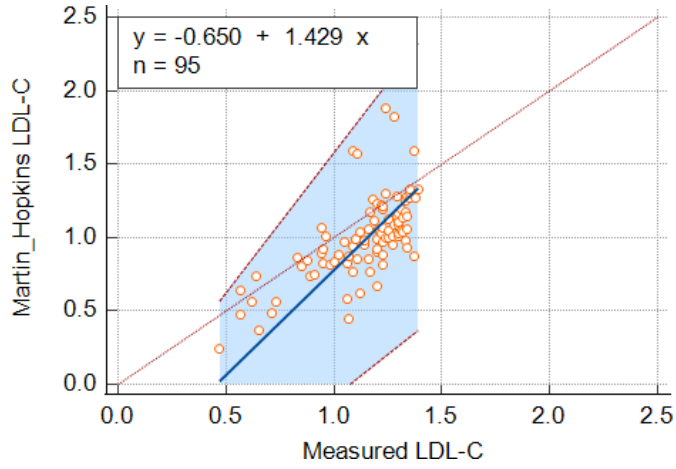
Appendix 1: Additional figures.

Supplementary Fig: Passing-Bablok plots at different low-density lipoprotein (LDL-C) treatment target concentrations using the Friedewald as well as the Martin-Hopkins formulae. (A-B) LDL-C of <1.4 mmol/L. (C-D) LDL-C of 1.4-1.7 mmol/L. (E-F) LDL-C of 1.8-2.5 mmol/L. (G-H) LDL-C of 2.6-2.9 mmol/L and (I-J) LDL-C ≥3.0 mmol/L. The plots show the regression line (Solid blue line) and the confidence interval for the regression line (dashed lines).

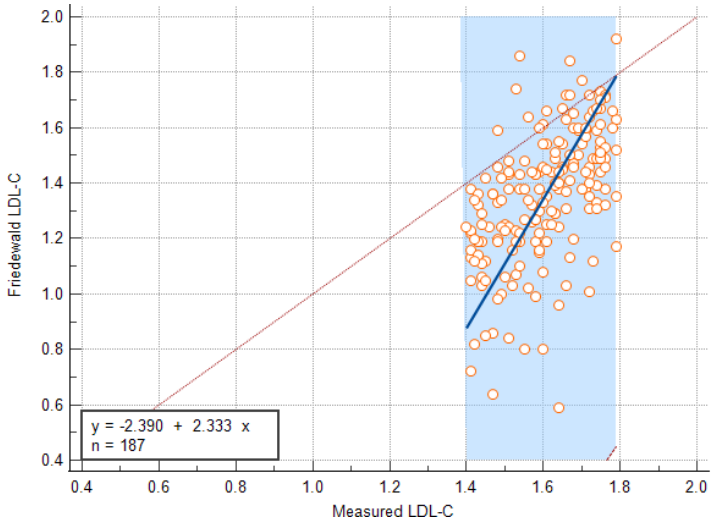
(A)



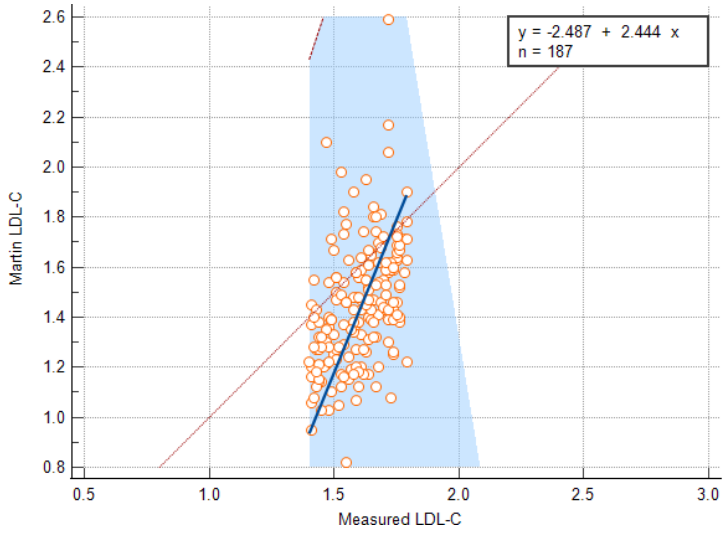
(B)



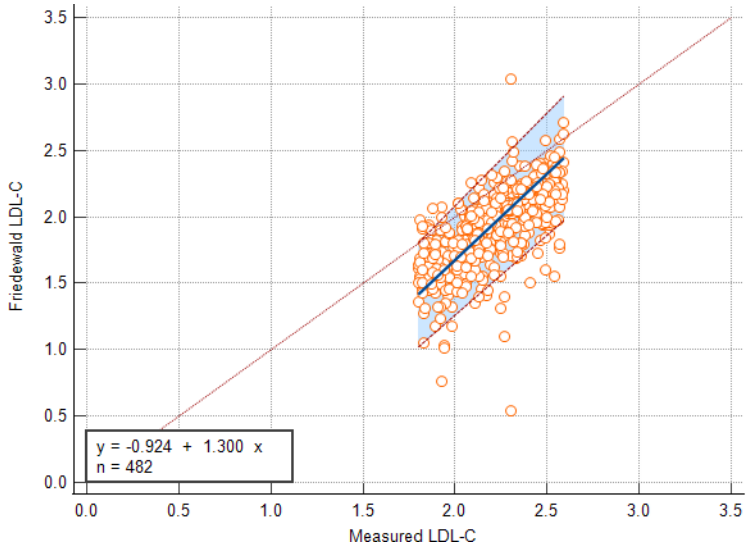
(C)


Friedewald LDL-C
Measured LDL-C
y = -2.390 + 2.333 x
n = 187
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0

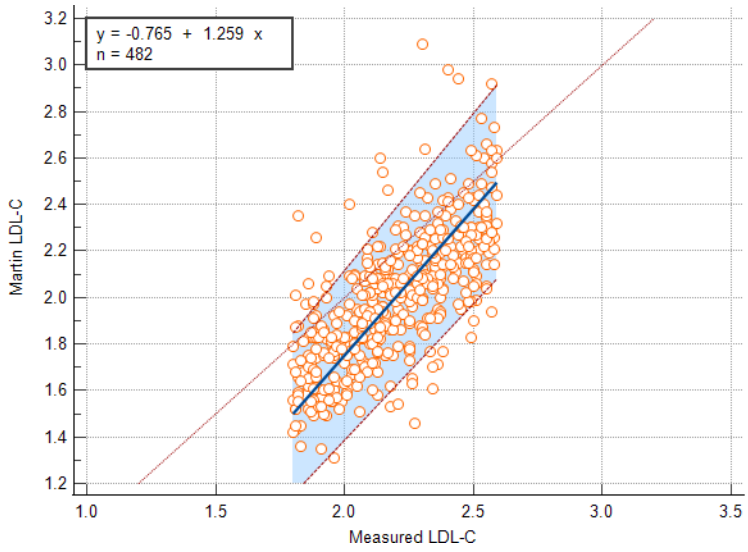
(D)


Martin LDL-C
Measured LDL-C
y = -2.487 + 2.444 x
n = 187
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.5
1.0
1.5
2.0
2.5
3.0

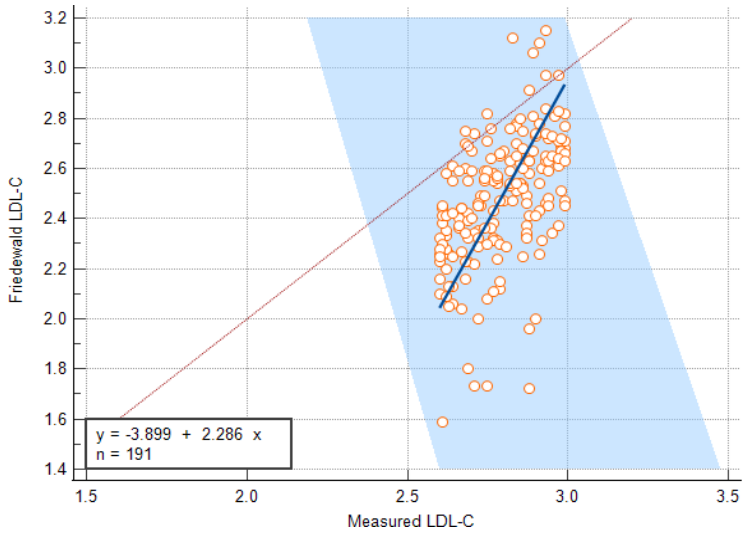
(E)


Friedewald LDL-C
Measured LDL-C
y = -0.924 + 1.300 x
n = 482
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

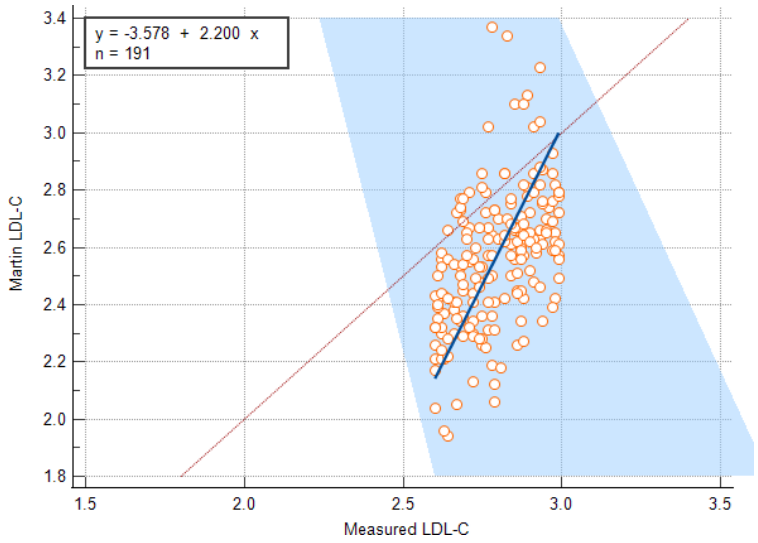
(F)


Martin LDL-C
Measured LDL-C
y = -0.765 + 1.259 x
n = 482
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
1.5
2.5
3.5

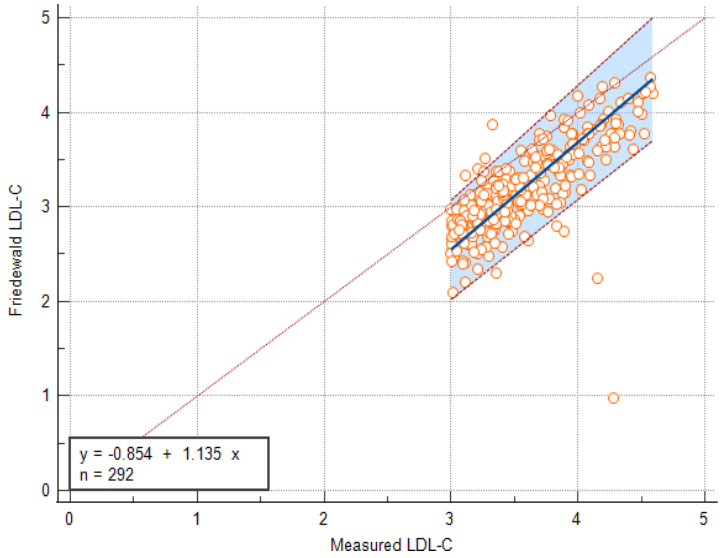
(G)


y = -3.899 + 2.286 x
n = 191
Friedewald LDL-C
Measured LDL-C
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.5
2.0
2.5
3.0
3.5

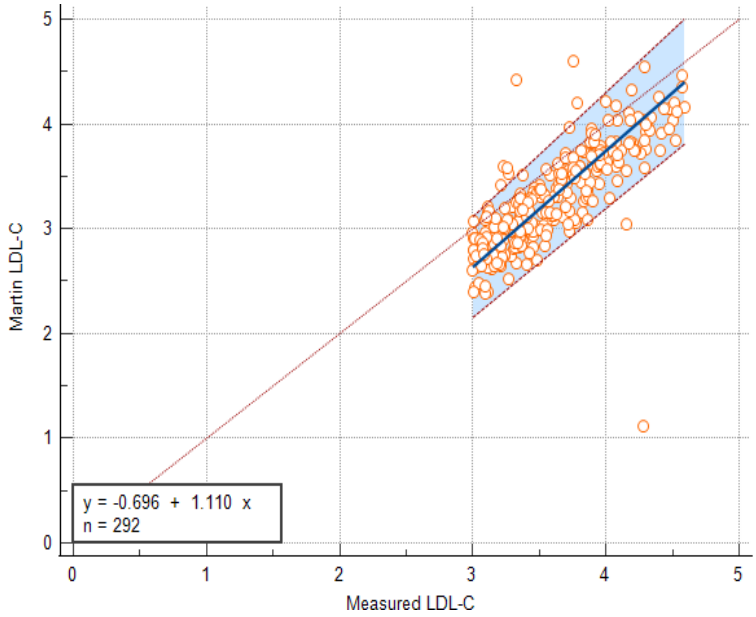
(H)


y = -3.578 + 2.200 x
n = 191
Martin LDL-C
Measured LDL-C
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.5
2.0
2.5
3.0
3.5

(I)


Friedewald LDL-C
Measured LDL-C
y = -0.854 + 1.135 x
n = 292
0
1
2
3
4
5

(J)


Martin LDL-C
Measured LDL-C
y = -0.696 + 1.110 x
n = 292
0
1
2
3
4
5

Appendix 2: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Postgraduate Affairs

TO: Drs MP Dintshi and N Kone
School of Pathology
Department of Chemical Pathology
Medical School

E-mail: mogomotsi12@gmail.com

CC: Supervisor: Dr S Khoza
Siyabonga.Khoza@nhls.ac.za
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 24 November 2020

REF: R14/49

PROTOCOL NO: **M200858** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Comparison of measured LDL cholesterol with calculated LDL cholesterol, using the Friedewald and Martin-Hopkins formulae in diabetic adults in Charlotte Maxeke Johannesburg Academic Hospital /NHLS Laboratory*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

R14/49 Drs MP Dintshi and N Kone

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200858

NAME: (Principal Investigator)	Drs MP Dintshi and N Kone
DEPARTMENT:	School of Pathology Department of Chemical Pathology Medical School University
PROJECT TITLE:	Comparison of measured LDL cholesterol with calculated LDL cholesterol, using the Friedewald and Martin-Hopkins formulae in diabetic adults in Charlotte Maxeke Johannesburg Academic Hospital /NHLS Laboratory
DATE CONSIDERED:	28 August 2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr S Khoza
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	24 November 2020

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore reports and re-certification will be due early in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

8/12/2020
Date