Supplemental Table 1: Summary of established and novel formulae to calculate LDL-cholesterol

Author (ref)	Year	Setting	N	Exclusions	Patients	Fasting status	Limitations	Formula	Performance
Friedewald [1]	1972	United States	448	TG>4.5 mmol/L Non-fasted patients	Familial Hyper-Cholesterolaemia	Fasted 12-14h	TG>4.5mmol/L LDL<1.8mmol/L	LDL-C = TC − HDL-C − TG/2.2 (mmol/L)	High correlation coefficients ranging from 0.94 to 0.98 (healthy population) and 0.99 (patients with dyslipidaemia)
DeLong [2]	1986	North America	>10000	Not available	Not available	Fasted ≥ 12hrs	Not available	LDL-C = TC − (HDL-C + 0.37 × TG)	Not available
Rao[3]	1988	Kuwait	196	None	Serial Lipid profiles	Unknown	None	([HDL-C × (a − c)] + (TC × c) – TG) / (c − b) A= HDL: TG/TC ratio = 0.15 B= LDL: TG/TC ratio = 0.095 C= VLDL: TG/TC ratio = 2.1 Ratios are determined using results from ultracentrifugation fractions	Linear correlation coefficients ranged between 0.93 – 0.937

Hattori [4]	1998	Japan	2179	TC<2.5 and >9 mmol/L and and TG<0.3 and >4.5 mmol/L Apo B <0.7umol/L	2161 out- and inpatients and 18 healthy controls	Fasted overnight	TC>9mmol/L and hyper-triglyceridaemia	LDL-C = (0.94TC − 0.94HDL-C − 0.19TG)	(r = 0.86) Between estimated and measured LDL was observed.
Puavilai [5]	2004	Thailand	1079	NA	Serum samples	Fasted	NA	LDL-C = TC − HDL-C − (TG/6)	Friedewald (72.3%) vs Puavillai (91.6%)
Anandaraja [6]	2005	India	2008	Not stated	Blood samples-lipid analysis (Indian patients)	Unknown	None	LDL-C = (0.9TC) − (0.9TG/5) – 28	d-LDL-C, LDL-C shows good correlation(r=0.97)
Teerakanchana [7]	2007	Thailand	1016	None	Outpatients Aged 8-89	Fasted 12-14 hrs	None	LDL-C = 0.91TC − 0.634(HDL-C) − 0.111TG − 6.755	Max correlation coefficient r=980

Ahmadi [8]	2008	Iran	237	None	TC>6.46mmol/L	Fasted	None	LDL-C = (TC/1.19) − (HDL-C/1.1) − (TG/1.9) – 38	No significant difference between direct and Ahmadi methods.
Chen [9]	2010	China	2180	<4.5 mmol/L	Outpatients >18 years	Fasted overnight	<4.5mmol/L	LDL-C = 0.9(Non-HDL-C) − 0.1TG (mg/dL)	Improved vs FW
Vujovic [10]	2010	Serbia	2053	TG>4.5 mmol/L	Routine follow up patients	Fasted overnight	TG >4.5 mmol/L	LDL-C = TC − TG/3 − HDL-C (mmol/L)	No significant difference between Vujovic LDL-C and direct-LDL was found.
De Cordova [11]	2013	Brazil	10664	None	Patients between 1-93 years old	Fasted 12 hrs	Not stated	LDL-C = 0.75(TC − HDL-C)	Higher correlation with directly measured LDL compared with Friedewald

Martin/ Hopkins [12]	2013	United States	1350908	None	Children adolescents and adults	Unknown	TG<4.5 mmol/L	LDL-C = TC – HDL-C – TG/adjustable factor	MH>FW for TG >4mmol/L and LDL <1.8 mmol/L vs dLDL
Sampson [13]	2020	United States	8656	Results <1st and >99th percentiles for each lipid parameter	Fasting patients at the NIH clinical centre	Unknown	TG > 9 mmol/L	LDL-C (mg/dL) = TC/0.948 – HDL-C/0.971 – (TG/8.56 + TG × non-HDL-C/2140 – TG^2/16100) – 9.44	35% less misclassifications in patients with hypertriglyceridemia.
Dudi [14]	2022	Sub-Himalayan region	1851	TG > 400mg/dL / 4.5 mmol/L	Fasting lipid profile in a Sub-himalayan population	Unknown	Does not account for general population and dylipidaemia	LDL-C = -2.421 + (0.752 × TC) – (0.047 × TG) – (0.350 × HDL-C)	Showed a better diagnostic performance as compared to other 10 equations taking Direct LDL-C as gold standard for Sub-himalayan Population and may be used as a substitute for FW in the study population.
Sampson [15]	2022		24713	TG > 3000 mg/dL / 33.87 mmol/L (N = 38) TG > 1500 mg/dL / 16.9 mmol/L (N = 3) Samples with detectable	Dyslipidaemic patients		An additional apoB test is required Equation only compared the original Sampson equation and eS-VLDL-C to F-LDL-C and did	eS-VLDL-C = (non-HDL-C/3.81) – (HDL-C/8.93) + (TG/7.73) + [(non-HDL-C × TG)/2050] – (TG^2 /13300) – (ApoB/2.49) – [(ApoB × TG)/3550] + 7.46	At a cut-point of 0.194, the first method showed a modest ability for identifying HLP3 (sensitivity = 73.9%; specificity = 82.6%; and AUC = 0.8685) but was comparable to the existing dual lipid apoB ratio method.

		United States		lipoprotein – X on agarose gel electrophoresis (N = 126)		Unknown	not include other equations New equation not assessed in the routine clinical lab setting No variability in ethnic groups		The second method based on eS-VLDL-C showed much better sensitivity (96.5%) and specificity (94.5%) at a cut-point of 0.209. It also had an excellent AUC value of 0.9912 and was superior to the two other methods in test classification.

Supplemental Table 2: Comparison of novel LDL-C equations in different populations

Country/Region	Clinical interest group	Homogeneous assay used	Calculations used	Publication year	Conclusion
Brazil	Type II Diabetes	Unspecified	Friedewald, Martin/Hopkins, Sampson/NIH	2022	Martin/Hopkins and Sampson's equations show a similar accuracy for calculating LDL-C concentrations in patients with diabetes, and both equations were marginally better than the Friedewald equation [16]
China	Coronary artery disease	Selective solubilization method test kit: *Kyowa Medex*	Friedewald, Martin/Hopkins, Sampson/NIH	2022	Even though misclassification by the Martin/Hopkins and Sampson equations was present in approximately 20% of patients versus directly measured LDL-C, directly measuring LDL-C did not identify any more individuals with increased risk of all-cause mortality, recurrent myocardial infarction, and major adverse cardiovascular events [17]
Germany	Chronic kidney disease	LDL-Cholesterol Gen. 3 kit on the Cobas VR modular platform 8000 or 6000: *Roche Diagnostics*	Friedewald, Martin/Hopkins	2020	The Friedewald formula yields lower LDL-C concentrations in CKD than direct enzymatic measurements [18]
Ghana	Cardiothoracic disease	Chema LDL direct FL test: *Hospitex Diagnostics*	Friedewald, Anandaraja, Martin-Hopkins	2018	The modified LDL-C formula derived by this study was more accurate compared to Anandaraja and Friedewald and could serve as a substitute for direct LDL-C and Friedewald in this population [19]
Hungary	All patients	Direct homogeneous method Wako on AU5800: *Beckman-Coulter*	Friedewald, Martin/Hopkins	2020	LDL-C estimation using the Martin/Hopkins formula yields a more accurate LDL-C value than that calculated by the Friedewald formula in this population [20]

India	All patients	LDL-C plus 2nd generation assay: *Roche Diagnostics*	Friedewald, Martin-Hopkins	2021	Martin/Hopkins calculated LDL-C values showed better agreement with directly measured values, and less bias and percentage total error than those obtained by use of the Friedewald formula [21]
	Hyperlipidaemia	Enzymatic colorimetric assay with rapid reagent kit: *Agappe*	Friedewald	2021	Both direct and calculated methods of LDL estimation have their limitations [22]
	Type II Diabetes	AU 680 clinical chemistry analyzer: *Beckmann-Coulter*	Friedewald, Hattori, Anandaraja, Chen, de Cordova, Teerakanchana, Ahmadi, Delong, Rao, Martin/Hopkins, Puavilai, Hata, Vujovic	2022	In this population all formulae except Ahmadi significantly underestimated the LDL-C when compared with the direct assay. The newer Martin's formula appeared to more precisely calculate LDL-C when compared with the Friedewald formula [23]
Iran	Adults	Direct LDL-C kit from Pars Azmoon on Roche Hitachi 912 chemistry analyser: *Roche Diagnostics*	Friedewald, Hattori, Anandaraja, Chen, de Cordova, Teerakanchana, Ahmadi, Delong, Rao, Puavilai Vujovic	2020	The Friedewald formula can be used in serum samples with TG up to 400 mg/dL when validated methods with correct calibration and control systems are used to derive the formula's componetns [24]
	Children	Enzymatic method on Auto Analyzer: *Hitachi*	Friedewald, Ahmadi, Chen, Anandaraja	2020	Friedwald had the strongest correlation with the direct assay and the Ahmadi formula the weakest at TG cut-ff of 100 mg/dL [25]
Israel	PCSK-9 inhibitors	N/A	Friedewald, Martin/Hopkins	2021	In high-risk patients achieving low LDL-C on PCSK9i treatment, Martin/Hopkins displayed significant proportion of LDL-C upward discordance compared to the Friedewald

					equation, identifying patients that may need treatment intensification [26]
	Coronary artery disease	N/A	Friedewald, Martin/Hopkins, Sampson/NIH	2020	Both Martin/Hopkins and Sampson/NIH LDL-C estimates displayed significant proportion of upward discordance compared to Friedewald formula, particularly in patients with elevated triglycerides and low LDL-C [27]
Italy	Children	Colorimetric Enzymatic Selective elimination assay: *Beckman-Coulter*	Friedewald; Sampson/NIH	2021	Sampson/NIH equation is more reliable than Friedewald [28]
	Hypertension	N/A	Friedewald, Martin/Hopkins, Sampson/NIH	2021	Higher LDL-C control rates were achieved using the Martin/Hopkins formula [29]
Japan	Type II Diabetes, Hypertriglyceridaemia	Unspecified	N/A	2022	The TG/LDL-C ratio is a reliable surrogate lipid marker of small dense LDL-C and superior to non–HDL-C in type 2 diabetes patients not treated with statins [30]
Korea	All patients	Pureauto S Cholestest-LDL on Automatic Analyzer 7600: *Hitachi*	Friedewald	2020	The Friedewald equation tends to underestimate LDL-C in high-risk subjects such as hypertriglyceridemia and hypo-HDL-cholesterolemia [31]
	Adults	Reagents from Sekisui, Beckman, or Roche Diagnostics on Hitachi 7600, Modular D2400, Beckman AU5800 or Abbott Architect Ci8200 automated analysers	Friedewald, Martin/Hopkins, Sampson/NIH	2021	Martin/Hopkins may be a readily adoptable and cost-effective alternative to direct LDL-C measurement, irrespective of dyslipidaemia status [32]

	Adults	LDL-cholesterol plus 2nd generation reagent and LDL-cholesterol Gen.3 on Cobas 8000 c702: *Roche Diagnostics*	Friedewald, Hattori, Anandaraja, Chen, De Cordova, Ahmadi, Delong, Rao, Puavilai, Martin/Hopkins, Vujovic	2021	The accuracy of 12 equations for LDL-C varied by cohort and subgroup based on direct-LDL and triglycerides [33]
	Atherosclerosis	Unspecified	Friedewald, Martin/Hopkins	2018	The Martin/Hopkins equation showed a 2-fold better concordance with LDL-C measured with a direct homogeneous assay in coronary atherosclerosis compared to the Friedewald equation when the TG level was ≥200 mg/dL [34]
Lebanon	All patients	Colorimetric assay after precipitation of non-LDL lipoproteins on 5600 dry chemistry analyzer: *VITROS*	Friedewald, Martin/Hopkins, de Cordova, Sampson/NIH	2022	A machine learning algorithm using K nearest neighbours is promising for the estimation of LDL-C as it agrees better with directly measured LDL-C than equations, especially in mild and severe hypertriglyceridemia [35]
Nigeria	Adults	Unspecified	Friedewald, Martin/Hopkins	2021	The Friedewald equation results in a lower LDL-C than the Martin/Hopkins formula [36]
North America	Adults	N/A	Friedewald, Martin/Hopkins	2021	The Martin/Hopkins equation correlates more strongly with non-HDL-C and ApoB than Friedewald and Friedewald underestimates LDL-C at low concentrations [37]
	PCSK-9 inhibitors	N/A (beta-quantification used)	Friedewald, Martin-Hopkins, Sampson/NIH	2021	LDL-C calculated by any of the three methods can guide treatment decisions for most patients, including those treated with proprotein convertase subtilisin/kexin type 9 inhibitors [38]
	Hypertriglyceridaemia	N/A (Vertical Auto Profile ultracentrifugation used)	Friedewald, Martin/Hopkins, Extended Martin/Hopkins, Sampson/NIH	2021	The extended Martin/Hopkins equation offered greater LDL-C accuracy compared with the Friedewald and Sampson/NIH equations in patients with triglyceride levels of 400 to 799 mg/dL [39]

	Adults	N/A	Friedewald, Martin/Hopkins, Sampson/NIH	2021	The Martin/Hopkins Sampson/NIH equations did not over- or under-estimate ASCVD risk compared to Friedewald in primary prevention, while Friedewald under-estimated subclinical ASCVD risk in the low-risk population. Martin/Hopkins or Sampson/NIH should be used for lipid screening in the general population [40]
	Type II Diabetes > 40 yrs	N/A	Friedewald, Martin/Hopkins	2020	The Martin/Hopkins equation for estimating LDL-C is a significant improvement on the Friedewald formula but remains an imperfect tool to estimate the atherogenic load in patients with high TG levels. Concordance with the alternate target parameters (apoB and non-HDL-C) was higher for Martin/Hopkins than for Friedewald when triglycerides levels were increased [41]
	Hypertriglyceridaemia in adults	Enzymatic colorimetric assay on 8000 Chemistry analyzer: *Roche Diagnostics*	Friedewald; Martin/Hopkins, Sampson	2022	Martin/Hopkins reduces LDL-C treatment group miscategorization rate leading to fewer underestimations of risk overall compared to Sampson/NIH; however, neither may be sufficiently accurate to report LDL-C in patients with TG ≥4.52 mmol/L (≥400 mg/dL) [42]
	Females with HIV	2-phase enzymatic assay after selectively solubilizing and enzymatically digesting non-LDL lipoproteins: *Quest diagnostics*	Friedewald, Martin/Hopkins, Sampson/NIH	2021	All 3 calculation methods have lower accuracy in women with HIV, even after adjusting for TG levels and fasting status [43]
	Males with HIV	N/A	Friedewald, Martin/Hopkins	2020	The Martin/Hopkins equation is more accurate than the Friedewald equation, especially at higher triglyceride levels, which are more common in people with HIV [44]

	Dyslipidaemia	N/A (beta-quantification used)	Modified Sampson/NIH	2022	The Sampson/NIH equation modified to include apoB, is nearly as accurate as the beta quantification reference method for the diagnosis of hyperlipoproteinaemia type 3 [15]
Slovakia	All patients	N/A	Friedewald, Martin/Hopkins, Sampson/NIH	2021	The results of clinical and epidemiological studies are significantly influenced by the method used to determine LDL-C [45]
South Africa	Outpatients	Automated direct enzymatic LDL-C: *Abbott Architect, Roche Cobas*	Friedewald, Hattori, Anandaraja, Chen, De Cordova, Sampson/NIH, Ahmadi, Delong, Martin/Hopkins, Puavilai, Vujovic	2021	Replacement of the Friedewald equation with Martin/Hopkins estimation to improve quality of LDL-C results can be safely implemented across analysers [46]
	Children Adults with diabetes	Automated direct enzymatic LDL-C: *Abbott Architect, Roche Cobas*	Friedewald, Hattori, Anandaraja, Sampson/NIH, Martin/Hopkins, Extended Martin/Hopkins	2022	Different measurement platforms influence the results of predictive equations and directly measured LDL-C: propose utilizing the Martin-Hopkins equation as an alternative to dLDL-C assays in adults with diabetes and for screening purposes in paediatric populations to avoid underestimating cardiovascular risk [94]
Sub-Himalayan	All patients	Direct LDL-C on AU5800 Clinical Chemistry Automated System: *Beckman-Coulter*	Friedewald, Puavilai, Vujovic, Anandaraja, Gowda, Teerakanchana, Ahmadi, de Cordova, Chen, Hattori	2022	The equation derived in this study showed a better diagnostic performance as compared to 10 other equations taking Direct LDL-C as the gold standard for Sub-Himalayan Population and may be used as a substitute for Friedewald [14]
Thailand	Adults	Unspecified	Friedewald, Ahmadi,	2021	Friedewald, Sampson/NIH, and Chen equations showed better estimations of LDL-C levels and

			Anandaraja, Chen, Cordova, Densethakul, Hattori, Martin, Puavillai, Sampson, Vujovic		had higher accuracy in classifying the degree of LDL-C strata [47]
Turkey	Cardiovascular disease > 40 yrs	N/A	Friedewald, Martin/Hopkins, Sampson/NIH	2021	In most cardiology outpatients, the Friedewald equation has excellent agreement with the Martin/Hopkins and Sampson equations [48]
	Adults with normotriglyceridaemia	2nd generation reagent on the 6000 c501 analyzer: *Roche Diagnostics*	Friedewald, Martin/Hopkins	2021	The Martin/Hopkins formula has good diagnostic compatibility with the direct LDL-C measurement [49]
Uganda	HIV > 50 yrs of age	Direct LDL-C on C311 chemistry analyzer: *Roche Diagnostics*	Friedewald	2021	Friedewald equation reliably calculated LDL-C [50]
United Kingdom	Type II Diabetics; Statins	LDL-C immunoassay on Synchron DxC analyser: *Beckman-Coulter*	Friedewald, de Cordova	2015	De Cordova agreed more closely with directly measured LDL-C than Friedewald [51]

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