Supplementary Online Content

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. *JAMA Cardiol.* Published online February 26, 2020. doi:10.1001/jamacardio.2020.0013

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Derivation of Equations

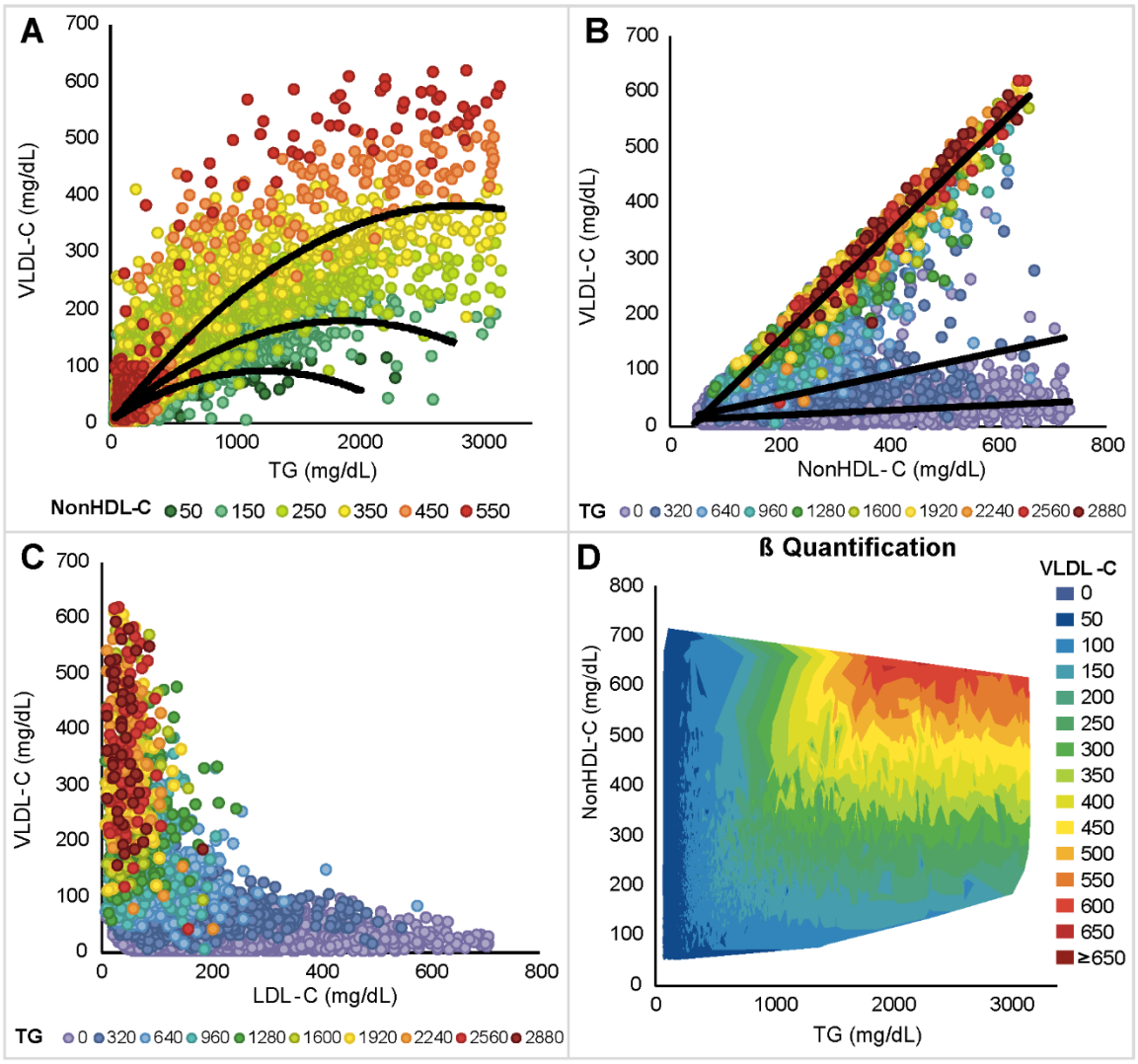
To gain insight into the best form of the equation for estimating VLDL-C, we plotted each independent variable (TG and nonHDL-C) against VLDL-C, using the NIH β-quantification database (eFigure 1). TG appeared to be linearly related to VLDL-C, but at higher TG values the curve flattened out (eFigure 1A). This was apparent when each individual point was color coded by nonHDL-C. Three curvilinear lines were fitted, using a second-order polynomial (see figure legend), for the three nonHDL-C groups indicated. When nonHDL-C was plotted against VLDL-C (eFigure 1B), a complex shape containing two arms was observed, again indicating the likely necessity of using at least a square function for one of the two independent variables. Based on color coding of the individual points, it appeared that for low TG values, VLDL-C was relatively independent of nonHDL-C but for higher TG values (≥320 mg/dL), VLDL-C increased almost linearly with nonHDL-C. Because nonHDL-C consists of two main components, namely LDL-C and VLDL-C, we plotted these two variables in eFigure 1C and color coded the individual points by TG. It can be seen from this plot that the shape of the curve in panel eFigure 1B is due to the inverse curvilinear relationship between VLDL-C and LDL-C. As TG increases, there is a sharp decrease in LDL-C and a corresponding increase in VLDL-C. Because apoB only slightly decreased with increasing VLDL-C, this relationship is likely due to enrichment of the core of LDL with TG by CETP-mediated lipid exchange. LDL-C appears to asymptotically approach a value of approximately 50 mg/dL as TG increases, which likely represents unesterified cholesterol on the outer phospholipid surface of the LDL particle.

In eFigure 1D, we made a contour plot for VLDL-C, using the two independent variables. Peak VLDL-C values occurred in the upper right corner of the plot, indicating an interaction between TG and nonHDL-C, which is also apparent from the Martin factor table. Overall, based on inspection of eFigure 1, it appeared that besides the two independent variables (TG and nonHDL-C), a square function of each variable and an interaction term involving their joint multiplication may be necessary to fit the surface described by the VLDL-C contour plot. These terms were entered into a multiple-regression model, but as indicated in eFigure 2A, only TG, TG^2 and (TG x NonHDL-C) terms were strongly predictive of VLDL-C and were then used to derive Equation 1 shown in the main text. In eFigure 2B, we show the value of each term of Equation 1 for various values of TG and nonHDL-C, which shows that because of its large denominator that the TG^2 term only becomes quantitatively important for high TG values. In eFigure 2C-E, we calculated VLDL-C by the Friedewald and Martin equations, as well as by Equation 1, for all possible values of TG and nonHDL-C and plotted it as a contour plot. By comparing the VLDL-C contour plot for eFigure 2C-E with the corresponding β-quantification VLDL-C contour plot in eFigure 1D, it is seen that Equation 1 better matches the empiric β-quantification VLDL-C results than the other equations. Similarly, as shown in eFigure 2F-H, the empiric factor value from Equation 1 better matches the empiric β-quantification factor value than the other equations.

Equation 2 was derived by taking the terms for VLDL-C in Equation 1 and subtracting it and HDL-C from TC as is done in the Friedewald equation. The coefficients for each term and intercept were calculated after multiple regression modelling for predicting β-quantification LDL-C and represent the mean of 10 random subsets of 50% of the training dataset.

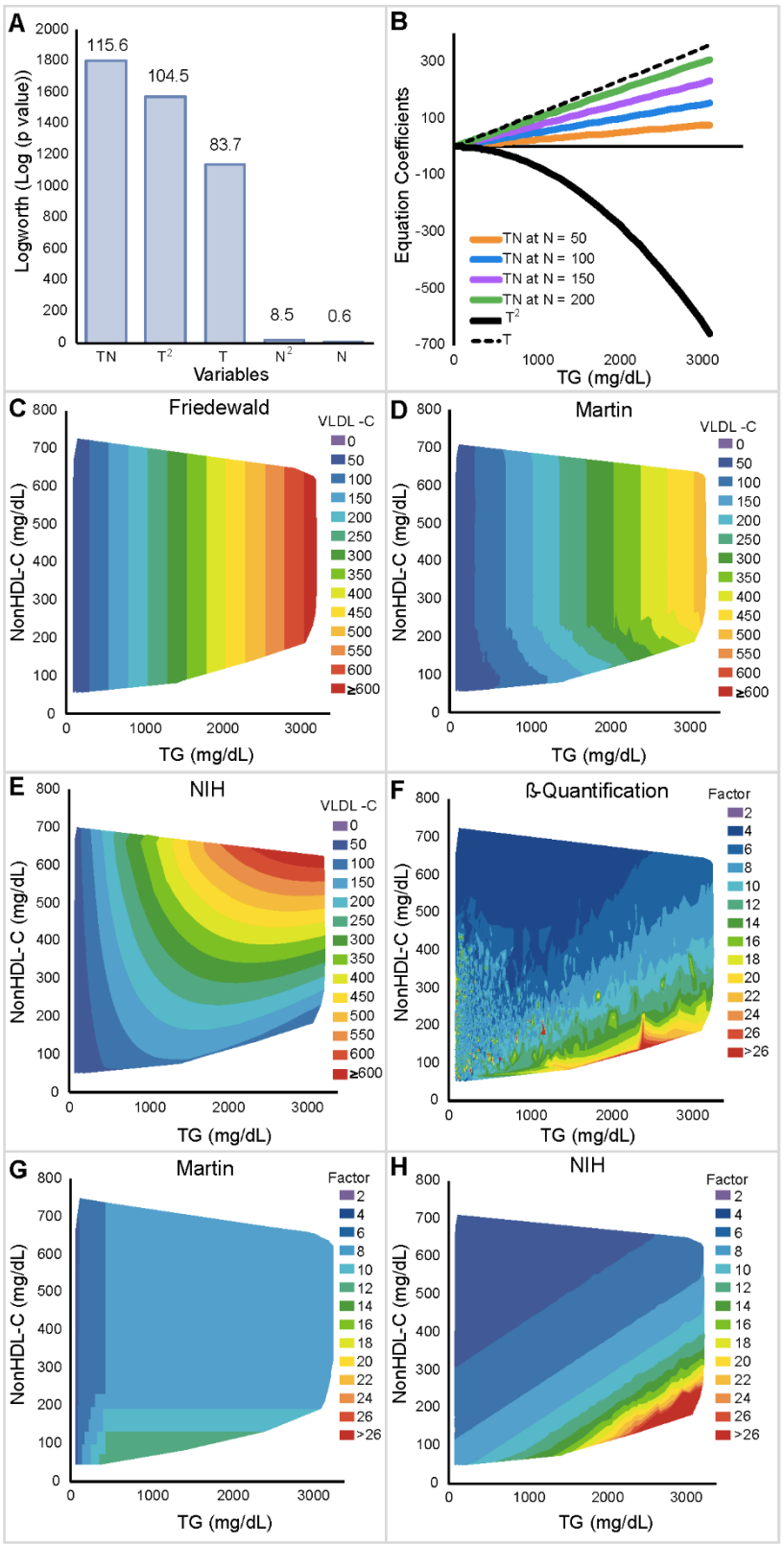
eAppendix 2. External Validation Datasets

Equations 1 and 2 were shared with laboratories with external datasets (Table 1) for evaluation. The first external β-quantification dataset (n=27,646) was from Mayo Clinic and included patients with dyslipidemia that were analyzed by β-quantification. The median TG was 127 mg/dL (IQR: 87-195) and 4.8% had TG>400 mg/dL. The median nonHDL-C was 140 mg/dL (IQR: 111-175). The second external β-quantification dataset (n=1245) was from Pacific Biomarker from a CETP-inhibitor clinical trial of patients also on a statin. The median TG was 98 mg/dL (IQR: 70-135) and 0.6% had TG>400 mg/dL. The median nonHDL-C was 99 mg/dL (IQR: 77-123). The first external dLDL-C dataset (n=231,682) was from LabCorp and included specimens from a general population tested for a standard lipid panel, which included the Roche dLDL-C test. The median TG was 127 mg/dL (IQR:88-194) and 6.6% had TG≥400 mg/dL. The median nonHDL-C was 128 mg/dL (IQR: 101-157). The second external dLDL-C dataset (n=21,206) was from Prism Health Dx Inc. and included specimens from a general population tested for the standard lipid panel which included the Beckman dLDL-C test. The median TG was 114 mg/dL (IQR: 79-171) and 5.3% had TG≥400 mg/dL. The median nonHDL-C was 130 mg/dL (IQR: 103-160).

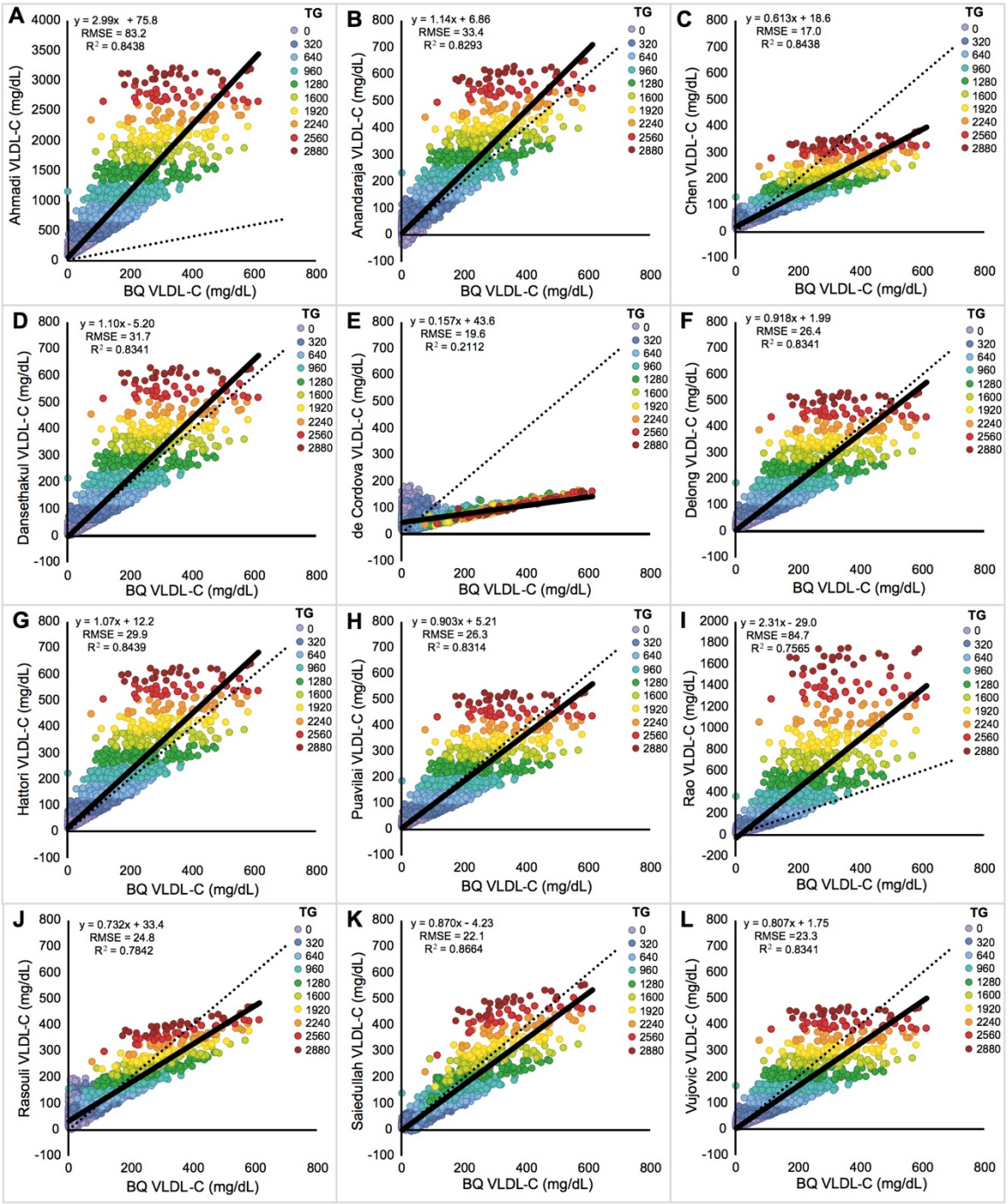


eFigure 1. Relationship of TG and non–HDL-C With VLDL-C. VLDL-C as measured by β-quantification is plotted against TG (A). Individual points are color coded by nonHDL-C. Solid lines indicate three second-degree polynomial regression equations ($-a*TG^2 + b*TG + k$) for how TG relates to VLDL-C for the following 3 nonHDL-C intervals: 53-125 mg/dL, 126-250 mg/dL, 251-734 mg/dL. VLDL-C as measured by β-quantification is plotted against non-HDL-C (B). Individual points are color coded by TG. Three linear regression lines for how nonHDL-C relates to VLDL-C are shown for the following 3 TG intervals: 38-99 mg/dL, 100-1499 mg/dL, 1500-3162 mg/dL. VLDL-C as measured by β-quantification is plotted against LDL-C (C).

Individual points are color coded by TG. VLDL-C as measured by β-quantification is plotted in a contour plot against both TG and nonHDL-C (D). Color codes corresponding to individual points indicate start of interval.

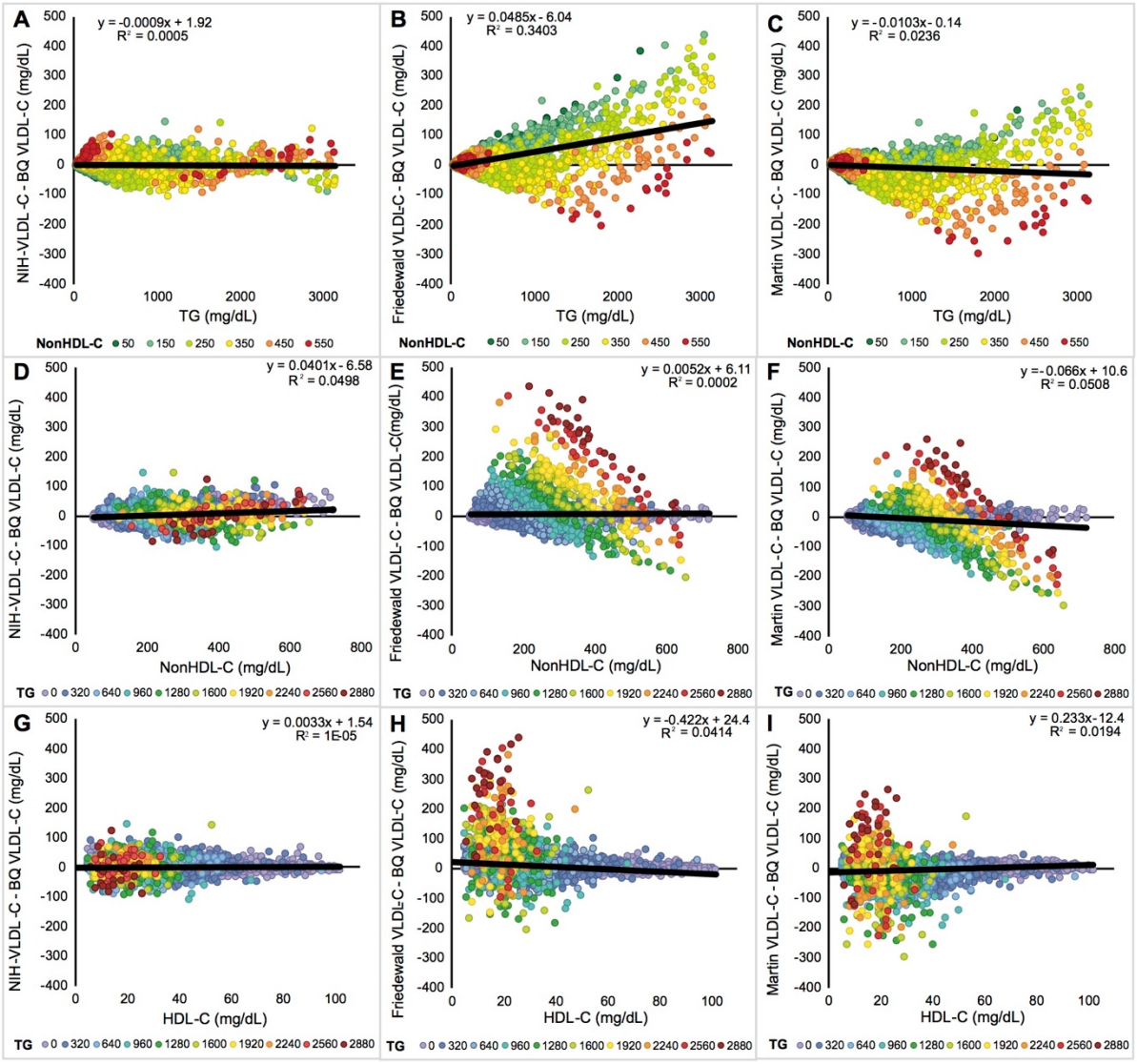


eFigure 2. Derivation of Equation 1. Panel A shows the logworth (log of the p-value) for the terms tested in Equation 1 (absolute value of the t-ratio is shown above each bar). (T=TG, N=nonHDL-C). The values for the individual terms for Equation 1 were calculated at the indicated fixed and variable levels of TG and nonHDL-C for each term of the equation (B). Contour plot shows VLDL-C level as represented by color code in mg/dL as calculated by the Friedewald equation (C), Martin equation (D) or Equation 1 (E) as a function of nonHDL-C and TG. Contour plots show the mean calculated factor level at various nonHDL-C and TG levels as determined for β-quantification (F), Martin equation (G) or Equation 1 (H). Color scale indicates factor level.

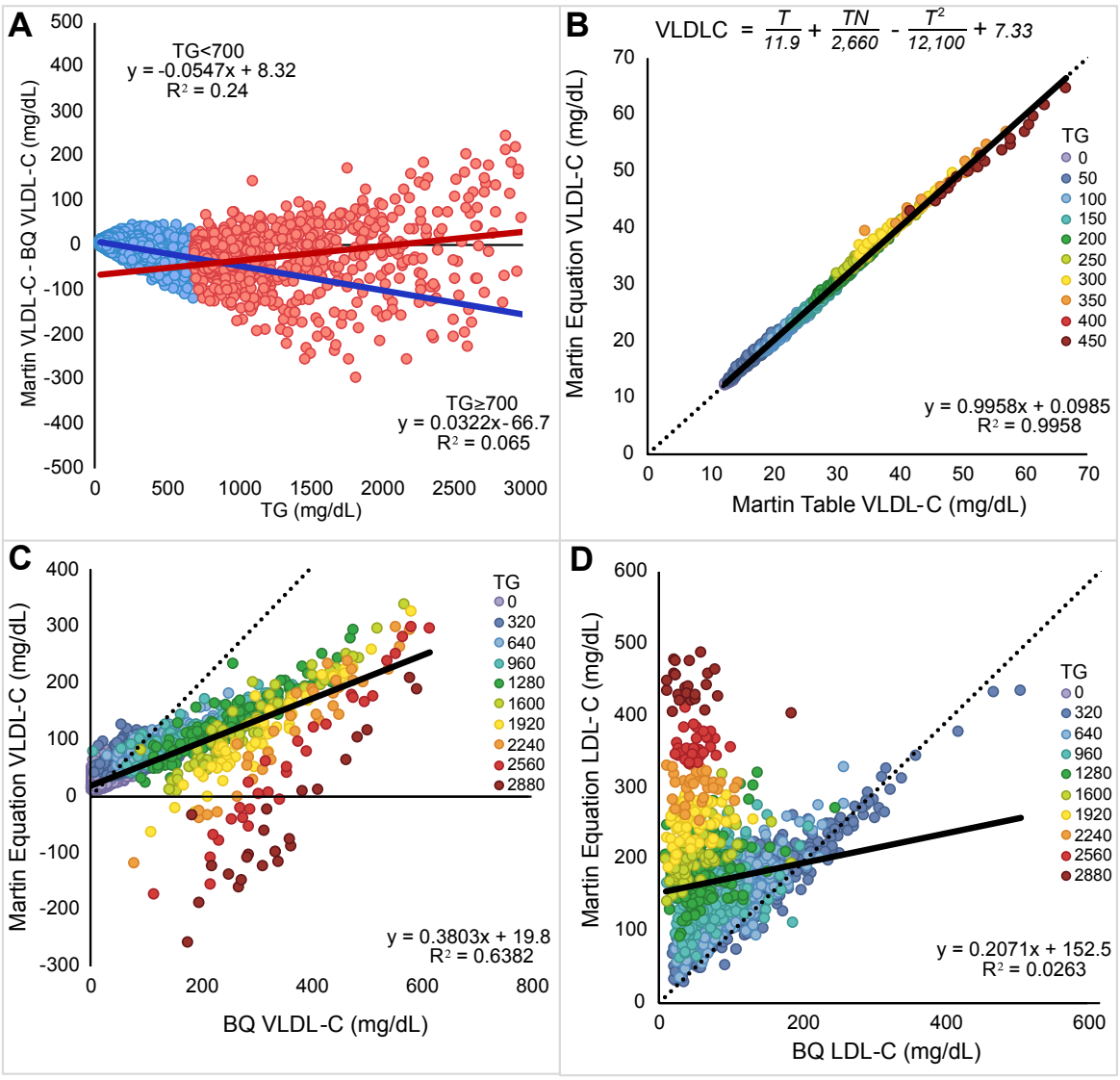


eFigure 3. Calculated Versus β-Quantification VLDL-C. VLDL-C was calculated with the indicated equation and plotted against VLDL-C as measured by β-quantification. Dotted line represents line of identity. Solid line is linear fit for indicated regression equation. RMSE and R^2

values are from the validation dataset (n=9,358). Points on the graphs indicating individual samples are color coded according to TG level. Color code for number scale for individual points indicates start of interval.

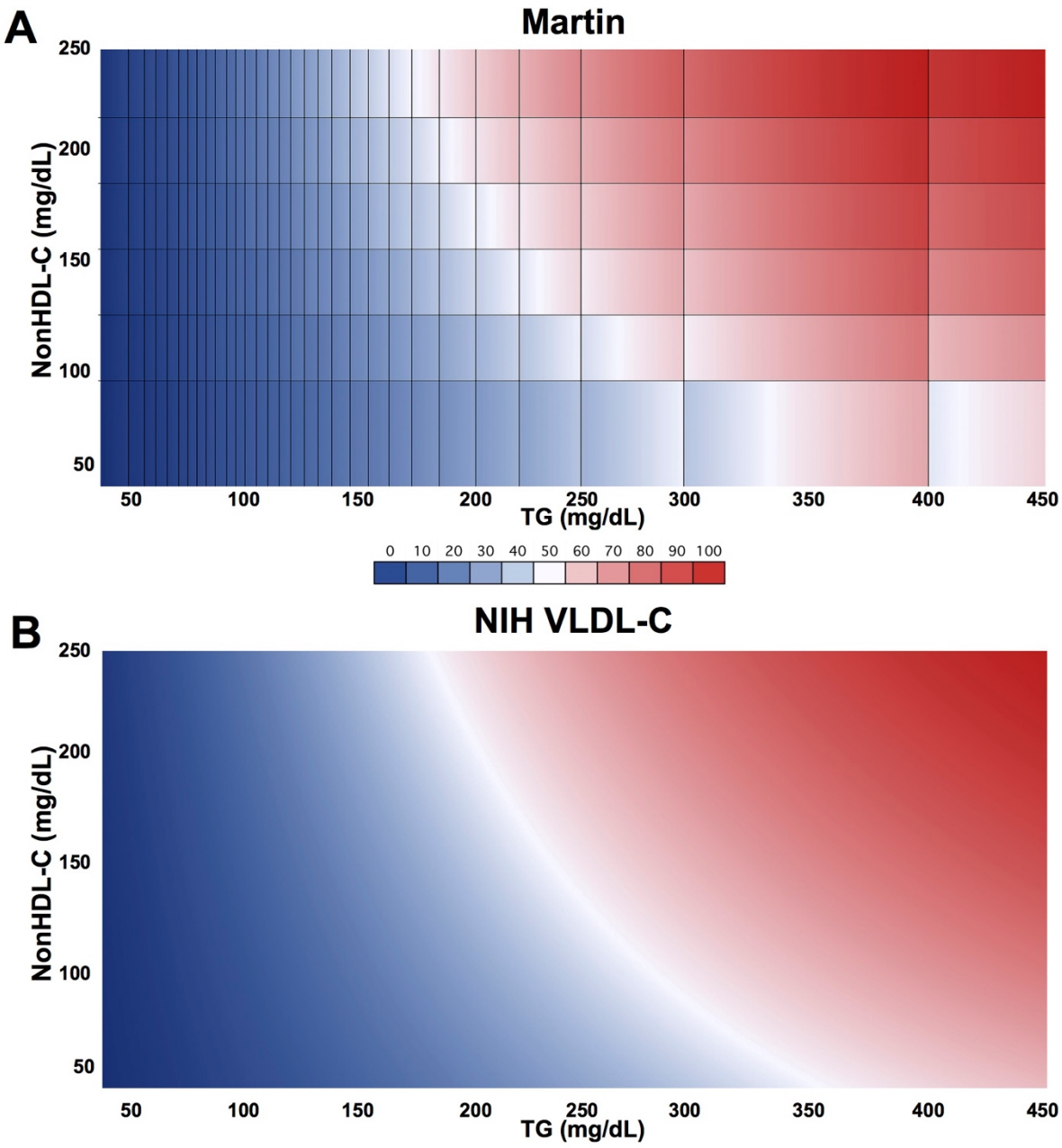


eFigure 4. Calculated Versus β-Quantification VLDL-C. Residual error plots were determined by the difference between calculated LDL-C and β-quantification test results for either Equation 1 (A, D, G), Friedewald equation (B, E, H) or Martin equation (C, F, I) and plotted against either TG (A-C), nonHDL-C (D-F) or HDL-C (G-I). Points on the graphs indicating individual samples are color coded according to either TG or nonHDL-C level as indicated. Color number scale for individual points indicates start of interval.

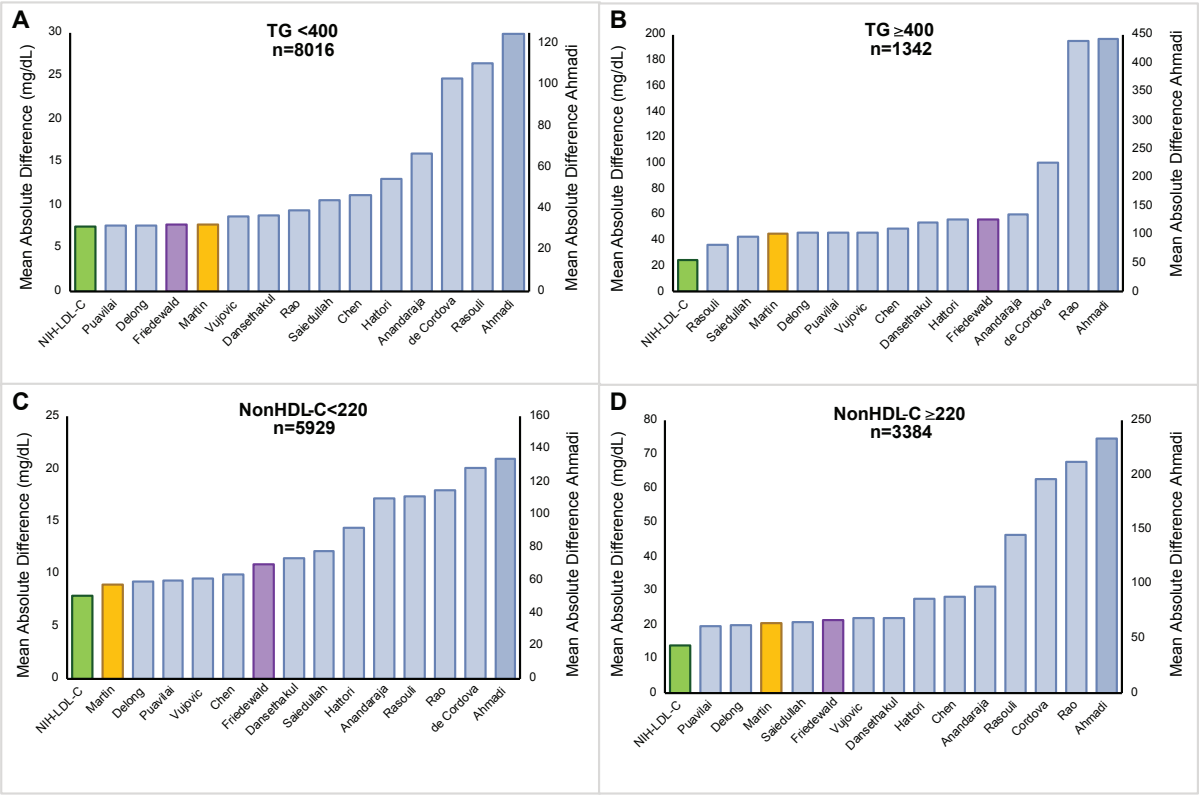


eFigure 5. Evaluation of Factor Table for Martin Equation. Difference between calculated VDL-C by Martin equation and β-quantification test results in validation dataset (n=9,358). Linear fit was performed separately for low-TG (TG<700 mg/dL, blue) and high-TG (TG≥700, red) samples (A). Factor values from the 2000-cell Martin factor table corresponding to TG<400 mg/dL and nonHDL-C<220 mg/dL were fitted by multiple-least squares regression analysis to the indicated equation. (T=TG, N=nonHDL-C) Results for VLDL-C using factor values from Martin factor table are on X-axis, whereas VLDL-C calculated using fitted equation based on

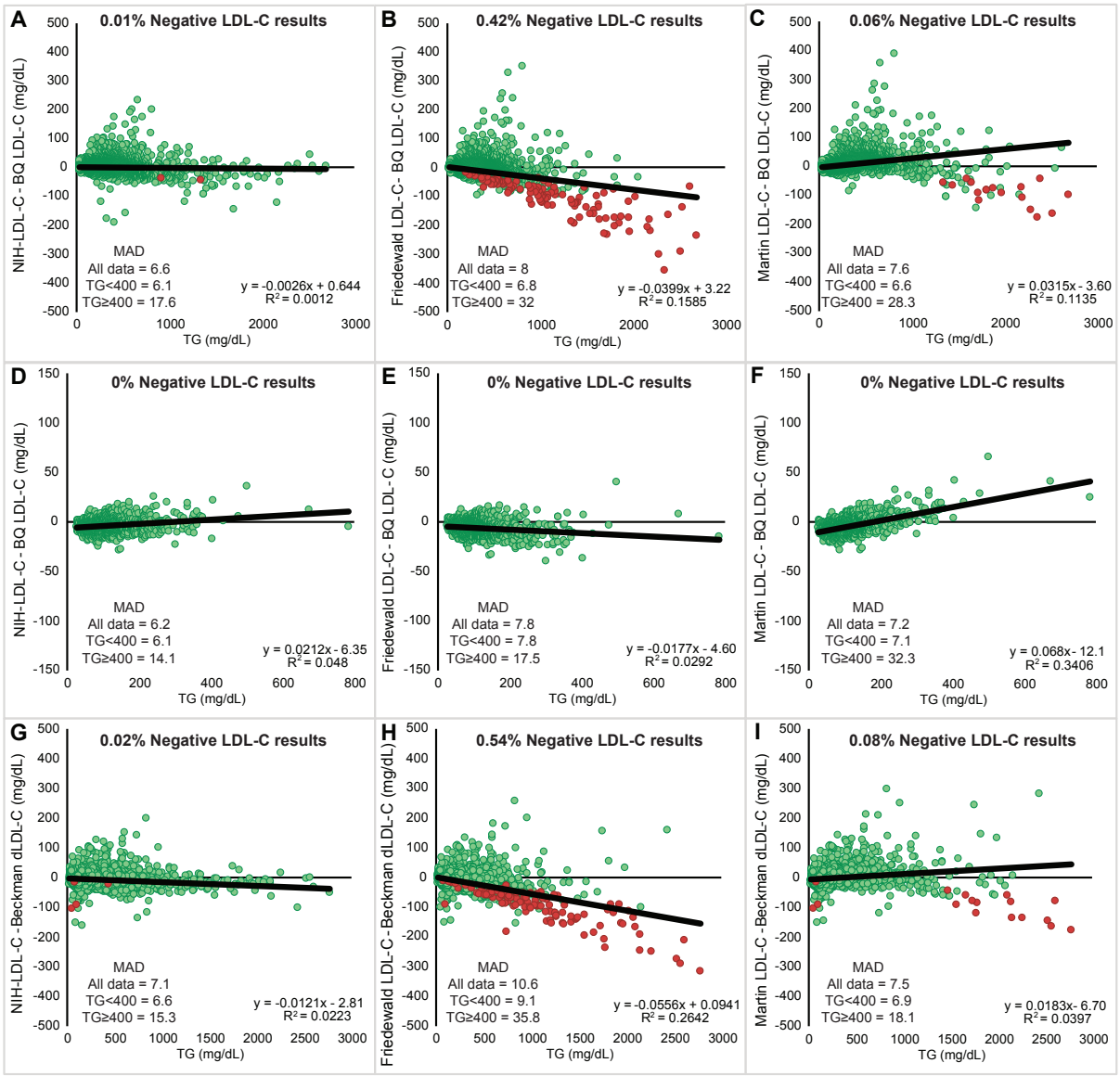
Martin factor table are shown on Y-axis. (B). VLDL-C was calculated with the fitted equation in Panel B and plotted against VLDL-C as measured by β-quantification (C). LDL-C was calculated by subtracting VLDL-C as calculated by the equation in Panel B from nonHDL-C and plotted against LDL-C as measured by β-quantification (D). Dotted line represents line of identity. Solid line is linear fit for indicated equation. Points on the graphs indicating individual samples are color coded according to TG level. Color code for number scale for individual points indicates start of interval.



eFigure 6. Contour plots for VLDL-C for Martin equation and Equation-1. Contour plots show VLDL-C in the area covered by the 180-cell Martin table for the Martin equation (A) or Equation 1 (B) as a function of nonHDL-C and TG. Individual black solid lines in Panel A indicate the individual data cells in the Martin table. Center scale indicates VLDL-C value in mg/dL.

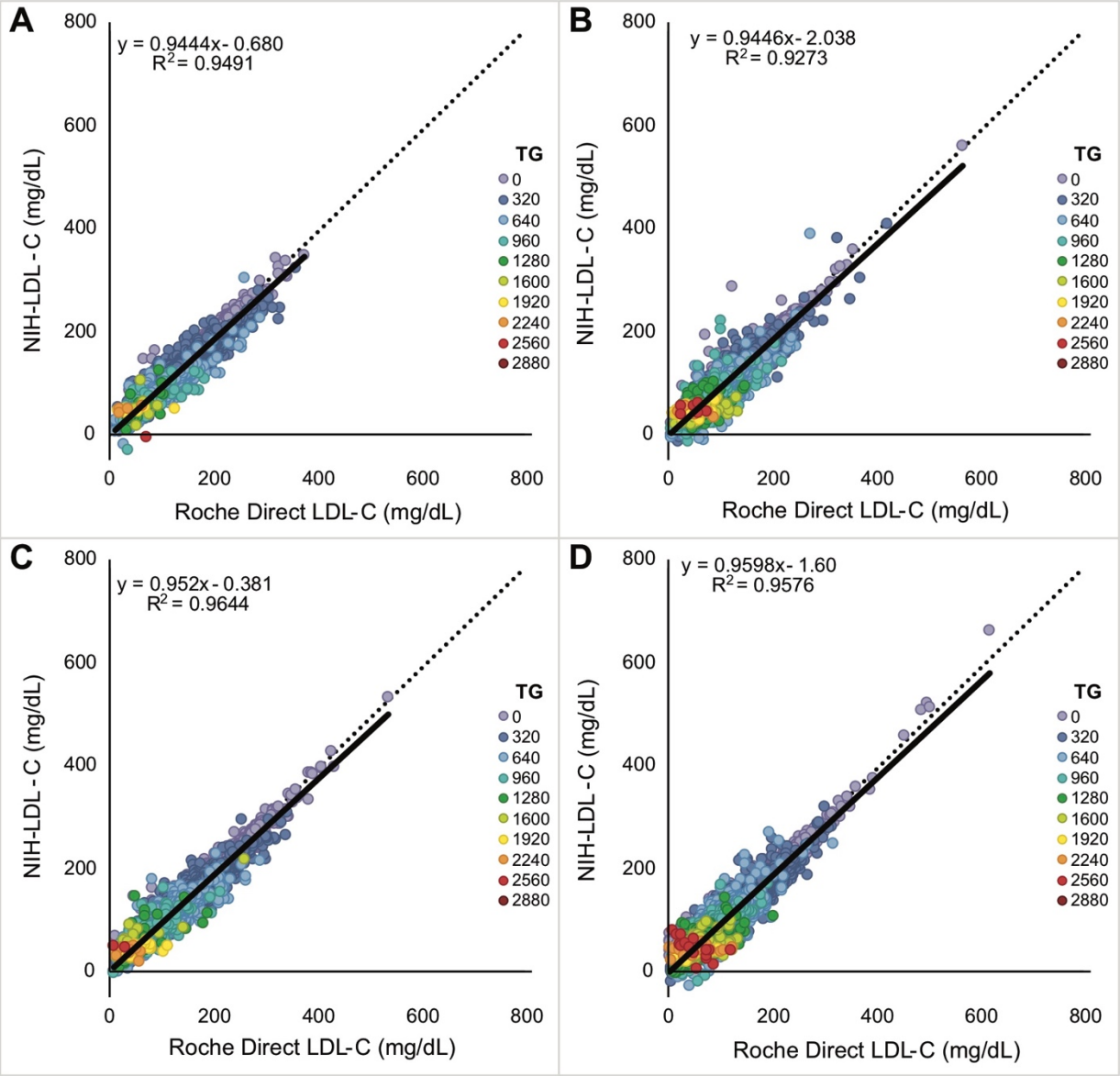


eFigure 7. MAD Score for LDL-C Equations. The mean absolute deviation (MAD) is calculated for indicated LDL-C equations and displayed in ascending order for TG<400 mg/dL (A), TG≥400 mg/dL (B), nonHLD-C <220 mg/dL (C) and nonHDL-C ≥220 mg/dL (D). Colors of individual bars correspond to either Equation 2 (green bar), Friedewald equation (purple bar) or Martin equation (orange bar). Other alternative equations are in pale blue bars. A secondary y-axis shows the MAD score for just the Ahmadi method, which was significantly higher than the other methods.

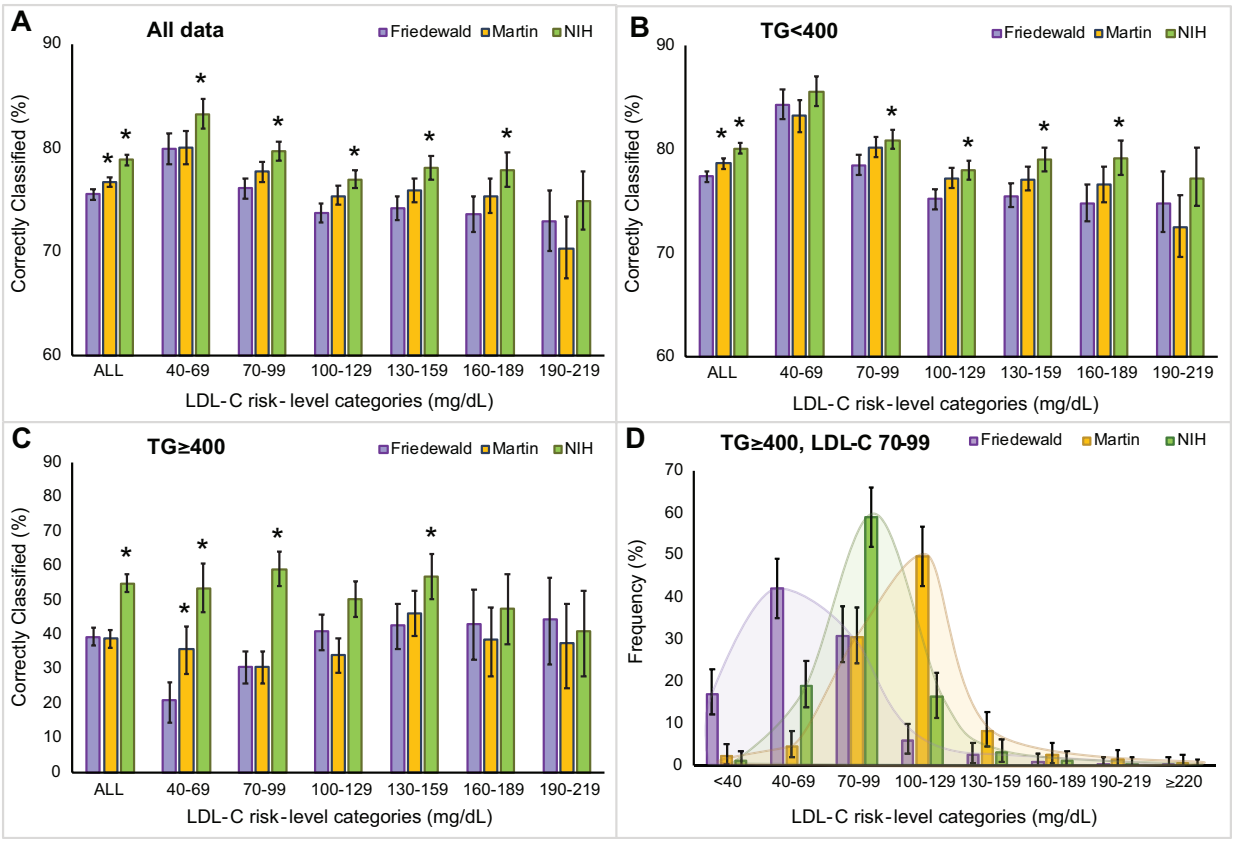


eFigure 8. Residual Error Plots for LDL-C by Various Equations. Difference between calculated LDL-C and β-quantification test results (n=27,646) for a general dyslipidemic population is shown for Equation 2 (A), Friedewald equation (B), and Martin equation (C) when plotted against TG. Difference between calculated LDL-C and β-quantification results (n=1245) for patients on statins plus/minus a CETP-inhibitor is shown for Equation 2 (D), Friedewald equation (E), and Martin equation (F) when plotted against TG. Difference between calculated LDL-C and direct LDL-C test results (Beckman, n=21,206) for patients from a specialty

reference laboratory is shown for Equation 2 (G), Friedewald equation (H), and Martin equation (I) plotted against TG. Negative LDL-C test results are shown as red dots, placed on top of non-negative test results (green dots) and hence make it appear that they are more abundant. Exact % of negative LDL-C test results are indicated at the top of each panel. The mean absolute deviation (MAD) for all indicated equations is shown in each panel for each dataset for either TG<400 mg/dL or TG≥400 mg/dL.



eFigure 9. Calculated Versus dLDL-C by Gender and Fasting State. LDL-C as calculated by Equation 2 was plotted against dLDL-C (Roche) for nonfasting females (A, n=29,373), nonfasting males (B, n= 28,130), fasting females (C, n=88,171) and fasting males (D, n=86,008). Dotted line represents line of identity. Solid line is linear fit for indicated regression equation. Points on the graphs indicating individual samples are color coded according to TG level (mg/dL). Color code for number scale for individual points indicates start of interval.



eFigure 10. Classification of Patients into LDL-C Risk-Level Categories. Concordance with β-quantification for classification of patients (n=27,646) into different LDL-C risk-level categories as defined by modified ATP-III criteria by the Friedewald equation (purple bars), Martin equation (orange bars) and Equation 2 (green bars) for complete database (A), TG<400 mg/dL (B), and TG≥400 mg/dL (C). Results are expressed as % of correctly classified cases by the various LDL-C equations when compared to β-quantification. Misclassification into other LDL-C risk-level categories (D) is shown for hypertriglyceridemic patients (n=362) with LDL-C between 70-99 mg/dL for the Friedewald equation (purple bars), Martin equation (orange bars) and Equation 2 (green bars). * indicates P<0.05 when compared to Friedewald equation.

eTable. Description of Existing Equations for Calculating LDL-C

Study	Equation	*N*	Population	LDL-C Method	Notes and Limitations
Ahmadi (18)*	$\frac{C}{1.19}-\frac{H}{1.09}-\frac{T}{1.9}-38$	230	Iran. Fasting samples, general population.	Direct method (Technicon)	Samples excluded: C> 250 mg/dL
Anandaraja (13)	$\frac{C}{1.11}-\frac{T}{5.55}-28$	1000	India. General population.	LDL-C precipitation heparin/sodium citrate	Not suitable for T> 350 mg/dL
Chen (19)	$\frac{N}{1.11}-\frac{T}{10}$	2180	China. General population.	Direct method (Roche)	Derived from samples with Tup to 1000 mg/dL
de Cordova (8)	$\frac{N}{1.33}$	10664	Southern Brazil. Fasting samples, general population.	Direct method (Wako)	T: 18 - 2574 mg/dL C: 73 – 523 mg/dL
Dansethakul (9)	$C-\frac{H}{1.01}-\frac{T}{5}+7.14$	1786	Thailand.	Direct method (Roche)	Derived from samples with Tup to 1000 mg/dL
DeLong (20)	$N-\frac{T}{6}$	6610	North America. Fasting samples, general population.	β–quantification	Derived from samples with Tup to 6000 mg/dL
Friedewald (2)	$N-\frac{T}{5}$	448	United States. Patients not on treatments for hyperlipoproteinemia.	β–quantification	Not suitable for T> 400 mg/dL
Hattori (16)	$\frac{N}{1.06}-\frac{T}{5.3}$	2179	Japan. General population.	β–quantification	Samples excluded: C> 350 or < 100 mg/dL; T> 400 or < 30 mg/dL; apoB <40 mg/dL
Martin (21)	$N-\frac{T}{AF}$	900605	United States. General population.	Vertical Auto Profile (Atherotech)	VAP under-recovers VLDL-C
Puavilai (14)	If T < 200 mg/dL: Friedewald Equation If T > 200 mg/dL: Delong Equation	999	Thailand. Fasting samples from general population.	Not stated	T< 300 mg/dL in study population
Rao (17)	$N-\frac{T}{4.9}+\frac{T^2}{9091}$	196	Kuwait. General population.	β–quantification	Not be suitable for T> 400 mg/dL
Rasouli (10)	$\frac{C}{1.33}-\frac{H}{2}-\frac{T}{10}$	300	Iran.	Direct method (Pars Azmon Inc)	Majority of sample population on statin therapy
Saiedullah (15)	$N-\frac{T}{5}+\frac{15.3\times T}{C-12.4}$	Unclear	Bangladesh.	Unclear	Details of derivation of equation unclear
Vujovic (7)	$N-\frac{T}{6.82}$	1010	Serbia. Fasting samples, general population.	Direct method (Kyowa Medex)	Samples excluded: T≥ 400 mg/dL

N– NonHDL cholesterol ($C-H$), T– Triglycerides, AF– Adjustable factor, C– Total cholesterol, H– HDL cholesterol
*References indicated in parenthesis