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Direct LDL Cholesterol Assay vs. Estimated Equations in Patients With Hypertriglyceridemia or Low LDL Cholesterol Levels

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Dear Editor,

Low-density lipoprotein–cholesterol (LDL-C) levels strongly correlate with cardiovascular disease risk. Guidelines recommend targeting low LDL-C levels to reduce this risk. Therefore, precise measurement is essential [1].

The LDL-C reference method requires ultracentrifugation by trained personnel and large serum samples [2, 3]. Equations, such as Friedewald, Martin–Hopkins, or Sampson, are often used instead to calculate LDL-C levels [4, 5]. Equations have limitations, particularly for the calculation LDL-C≤70 mg/dL or triglycerides levels (TG) ≥400 mg/dL. Furthermore, bias and imprecision from the different measurements used in the calculation can adversely affect LDL-C calculation accuracy [6].

LDL-C clinical decision limits are based on the Friedewald equation. Despite the additional cost, the direct LDL-C method (dLDL-C) is faster than ultracentrifugation and more accurate than equations. dLDL-C results may vary either among manufacturers or depending on the reagent-calibrator-instrument combination. Therefore, it is advisable to compare the dLDL-C with the reference method [7].

We assessed and compared the dLDL-C and equations for calculating LDL-C against the reference method to identify quick and accurate alternatives. This was a prospective study developed in Spain, from March 2022 to March 2023.

In total, 212 serum samples stored at 4°C were analyzed and categorized into two groups (IRB; PI-21-036): group 1 (TG≥400 mg/dL, positive lipemic index, N=113) and group 2 (TG≤200 mg/dL, LDL-C≤70 mg/dL, N=99). Group 1 was subdivided into 1a (TG=400–500 mg/dL, N=62) and 1b (TG=500–900 mg/dL, N=51). Group 2 was subdivided into 2a (LDL-C=10–40 mg/dL, N=49) and 2b (LDL-C=40–70 mg/dL, N=50).

dLDL-C level was measured using the enzymatic selective protection method [8] with an AU5800 analyzer (Beckman Coulter, Brea, CA, USA). The dLDL-C calibrator value is traceable to the reference method. LDL-C was calculated using the Friedewald, Martin–Hopkins (180-cell strata), and Sampson equations. Very-low-density lipoprotein–cholesterol (VLDL-C) was isolated from 1.5 mL of serum utilizing sequential density gradient ultracentrifugation at 100,000×*g* for 18 hrs with an F50L fixed-angle rotor (Thermo Scientific, Basingstoke, UK) using KBr for density adjustment (1,006 g/mL). TC, HDL-C, TG, and VLDL-C contents were measured using the AU5800 analyzer, and LDL-C level was

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calculated as follows: LDL-C = TC−HDL-C−VLDL-C [2, 3, 4].

Statistical analysis was conducted using MedCalc v19.6 (MedCalc Software, Ostend, Belgium). Means were compared using *t*-test for paired samples to compare the different methods against the reference method.

Bias between methods was calculated as follows:

$$Bias = (Cx - Cn)/Cn \times 100$$

where Cn and Cx represent the LDL-C determined using the reference and alternative methods, respectively. Bias was compared with the reference change value (RCV), which was calculated for LDL-C considering a unilateral Z statistic with 95% confidence (Z = 1.65), as follows:

$$RCV = Z \times 2^{1/2} \times (CVa^2 + CVi^2)^{1/2}$$

where CVa and Cvi are the analytical CV and within-subject biological variation according to the European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation Database, respectively [9]. The RCV was calculated to be 20.0% based on CVa = 2.18 and CVi = 8.3. Bland–Altman difference plots were used to compare the reference and alternative methods in groups 1 and 2.

Table 1 reveals significant differences in LDL-C between the reference and alternative methods, except for dLDL-C in group 1. However, the bias did not exceed the RCV for dLDL-C and Martin–Hopkins. In group 2, LDL-C differed significantly from those determined using the reference method for all alternative methods; however, the bias did not exceed the RCV for dLDL-C.

In groups 1a and 1b, LDL-C measured using the reference method differed significantly from the values determined using the alternative methods, except for dLDL-C. The bias never exceeded the RCV for dLDL-C in groups 1a and 1b nor for Martin–Hopkins in group 1a. In groups 2a and 2b, all methods differ significantly from the reference method; however, the bias did not exceed the RCV for dLDL-C (Table 1).

Bland–Altman plots demonstrated minimal bias of dLDL-C vs. the reference method in groups 1 and 2 (Fig. 1).

Notably, for TG ≥ 400 mg/dL, dLDL-C was the superior method.

Table 1. Comparison of methods for direct LDL-C assay and estimated equations for LDL-C with a reference method

Group	Variable	Ultracentrifugation*	dLDL-C	Friedewald	Martin–Hopkins	Sampson
Group 1 (TG ≥ 400 mg/dL)	Median (IQR)	148 (116–177)	149 (130–170)	85 (59–112)	121 (101–142)	101 (81–122)
	P		0.7745	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		0.5	−42.4	−18.5	−31.7
Group 1a (TG = 400–500 mg/dL)	Median (IQR)	151 (115–180)	149 (122–174)	93 (61–124)	126 (101–151)	106 (78–132)
	P		0.4070	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		−1.5	−38.4	−16.6	−30.1
Group 1b (TG = 500–900 mg/dL)	Median (IQR)	148 (124–174)	148 (132–165)	77 (55–98)	114 (102–134)	98 (82–112)
	P		0.8201	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		−0.2	−48.3	−22.7	−34.0
Group 2 (LDL-C ≤ 70 mg/dL)	Median (IQR)	58 (49–62)	61 (54–68)	41 (34–46)	45 (37–50)	43 (36–48)
	P		< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		5.3	−29.6	−22.9	−25.4
Group 2a (LDL-C = 10–40 mg/dL)	Median (IQR)	49 (44–54)	55 (51–63)	34 (29–38)	36 (30–41)	36 (29–40)
	P		< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		10.9	−31.8	−25.8	−26.7
Group 2b (LDL-C = 40–70 mg/dL)	Median (IQR)	62 (58–66)	65 (61–69)	46 (43–49)	49 (46–53)	48 (45–51)
	P		< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Bias (%)		5.8	−25.1	−20.2	−21.9

*Reference method.
Abbreviations: IQR, interquartile range; TG, triglycerides; LDL-C, low-density-lipoprotein cholesterol; dLDL-C, direct LDL-C assay.

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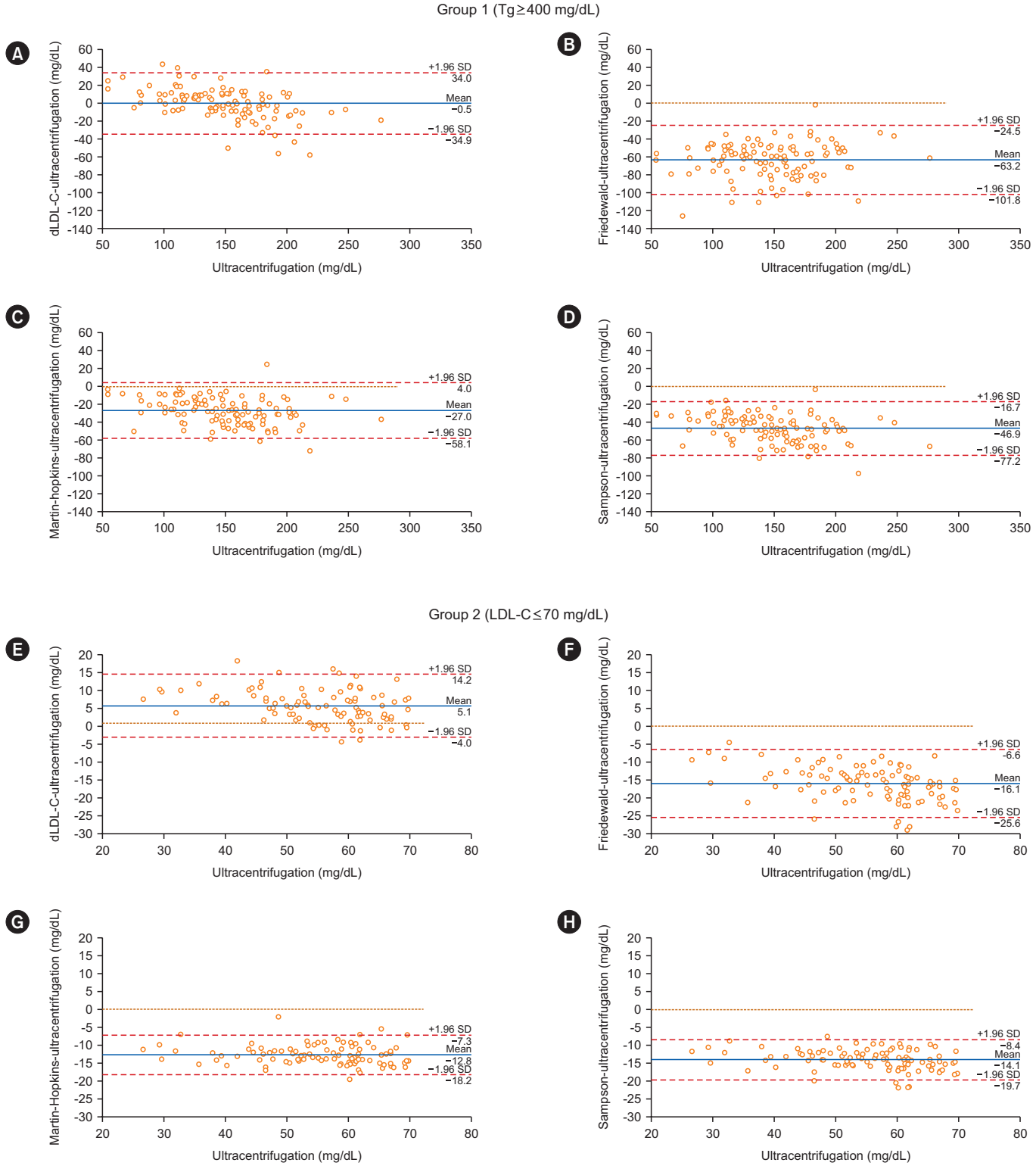

Fig. 1. Bland–Altman plots for the alternative methods and the ultracentrifugation (reference) method. (A) dLDL-C, (B) Friedewald, (C) Martin–Hopkins, and (D) Sampson methods applied in group 1. (E) dLDL-C, (F) Friedewald, (G) Martin–Hopkins, and (H) Sampson methods applied in group 2.
Abbreviations: TG, triglycerides; LDL-C, low-density lipoprotein–cholesterol; dLDL-C, direct low-density lipoprotein–cholesterol.

For TG = 500–900 mg/dL, results from all alternative methods differed from ultracentrifugation results, except for dLDL-C. For TG = 400–500 mg/dL, both dLDL-C and Martin–Hopkins methods were suitable, with dLDL-C being the most accurate. dLDL-C is applicable for LDL-C < 70 mg/dL.

For LDL-C < 70 mg/dL or TG ≥ 400 mg/dL, Friedewald, Martin–Hopkins, and Sampson tend to underestimate LDL-C. Martin–Hopkins and Sampson equations reportedly have improved accuracy over Friedewald with TG ≥ 400 mg/dL or LDL-C < 70 mg/dL [5, 10]. However, these studies did not compare these methods with ultracentrifugation, but with dLDL-C.

In conclusion, the study showed that both equations and the Friedewald method continue to underestimate LDL-C. The findings support that dLDL-C is an excellent choice for TG ≥ 400 mg/dL or LDL-C < 70 mg/dL. These results are specific for the Beckman Coulter dLDL-C; differences among manufacturers have been reported [7]. Similarly, TC, HDL-C, and TG assays used to calculate LDL-C are not perfectly standardized among manufacturers.

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AUTHOR CONTRIBUTIONS

Martínez-Bujidos M, Morales-Indiano C and Fernández-Prendes C contributed to the conception and design of the study; Rodríguez-Domínguez J, Piedra-Aguilera A, and Fernández-Prendes C interpreted the results; Piedra-Aguilera A performed the statistical analysis; Rodríguez-Domínguez J, Piedra-Aguilera A, Malumbres-Serrano S and Fernández-Prendes C drafted the manuscript; and Fernández-Prendes C supervised the study. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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