COMPARATIVE STUDY OF LDL-CHOLESTEROL LEVELS IN THAI PATIENTS BY THE DIRECT METHOD AND USING THE FRIEDEWALD FORMULA

Thirawuth Teerakanchana[1], Wilai Puavilai[1], Kanjana Suriyaprom[2] and Rungsunn Tungtrongchitr[3]

[1]Clinical Chemistry Laboratory, Department of Clinical Pathology, Rajavithi Hospital, Ministry of Public Health, Bangkok; [2]Faculty of Medical Technology, Rangsit University, Pathum Thani; [3]Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. In this study, low-density lipoprotein cholesterol (LDL-C) levels by direct measurement and estimation using the Friedewald formula, were compared among 1,016 Thai patients. The study assessed blood samples from out-patients sent to the Clinical Chemistry Laboratory, Department of Clinical Pathology, Rajvithi Hospital, Ministry of Public Health, for measurement of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels, January 2004-December 2005. Patients' ages ranged 8-89 years, 573 (56.4%) were females. Linear regression analysis showed the two methods had highly significant correlation coefficients ($p<0.001$). Upon comparing the two methods, at TG levels of 151-200 mg/dl, bias was 18.3 mg/dl; and for TG levels of 201-300 mg/dl, bias was lower at 11.4 mg/dl; for TG levels of 301-400 mg/dl, bias increased to 20.9 mg/dl. The direct assay meets currently established analytical performance targets and may be useful for the diagnosis and management of hyperlipidemic patients. The Friedewald formula did not give a homogeneous performance when estimating LDL-C levels in samples with different TG levels.

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of death worldwide. Cigarette smoking, high blood pressure, and increased low-density lipoprotein cholesterol (LDL-C) concentration are risk factors for the development of CAD (Scandinavian Simvastatin Survival Study Group, 1994; Sacks *et al*, 1996). According to the National Cholesterol Education Program-Adult Treatment Panel II (NCEP-ATP II), the diagnosis and management of patients with hypercholesterolemia is largely based on the concentration of LDL-C (NCEP II, 1994). NCEP-ATP II defines LDL-C values < 3.37 mmol/l (130 mg/dl) as "desirable" and those >4.14 mmol/l (160 mg/l) as "high". For patients with CAD, the tentative treatment goal is to lower LDL-C to 2.6 mmol/l (100 mg/dl) or lower (NCEP II, 1994), which is the same as the NCEP-ATP III (NCEP III, 2001) recommendations.

The reference method for determining LDL-C is β-quantitation (Bachorik, 1997), which requires ultracentrifugation of the samples. Therefore, this method is not suitable for routine laboratory testing. For that reason, most laboratories estimate LDL-C using the Friedewald formula (FF) (Friedewald *et al*, 1972), which calculates concentrations of total cholesterol (TC), cholesterol present

Correspondence: Dr Rungsunn Tungtrongchitr, Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.
Tel: 66 (0) 2-354-9100 ext 1582, 1584, 2089; Fax: 66 (0) 2-644-7934
E-mail: tmrtg@mahidol.ac.th

in high-density lipoproteins (HDL-C) and triglycerides (TG) (Friedewald *et al*, 1972). The Friedewald calculation for LDL-C is as follows: LDL-C = TC - (HDL-C) - (VLDL-C), where the very low-density lipoprotein cholesterol (VLDL-C) concentration is estimated from serum triglyceride concentration (in mg/dl) as VLDL-C = TG/5.

The formula assumes the ratio of TG to VLDL-C to be constant in all samples. However, there are some limitations to this postulation. For example, the formula can overestimate VLDL-C and underestimate LDL-C if triglyceride-rich chylomicrons and chylomicron remnants are present in the serum specimen (McNamara *et al*, 1990).

The National Cholesterol Education Program (NCEP) established that clinical laboratories should use methodologies for measuring LDL-C levels with a total analytical error <12%, imprecision <4%, and inaccuracy <4%, to guarantee correct patient classification in the NCEP risk categories (Cordova *et al*, 2004). It is difficult to obtain this analytical quality with Friedewald formula (FF) because each component's analytical error is added. The analytical performance of the direct LDL-C method needs to be assessed, so that it can be used routinely in clinical laboratories, and compared with the FF. Therefore, the aim of this study was to assess the analytical performance of an enzymatic determination of LDL-C compared with the FF.

MATERIALS AND METHODS

Samples

The study assessed blood samples of 1,016 out-patients which were sent to the Clinical Chemistry Laboratory, Department of Clinical Pathology, Rajavithi Hospital, Ministry of Public Health, Thailand, for TC, LDL-C, HDL-C and triglyceride measurements, January 2004-December 2005. The patients'ages ranged 8-89 years and 573 (56.4%) were females. Blood samples were collected after fasting 12-14-hours, allowed to clot at room temperature, thereafter serum was obtained by centrifugation at 2,000 round for 15 minutes. All direct analyses were performed on the same day.

The study protocol was approved by the Ethics Committee of Rajavithi Hospital, Ministry of Public Health, Bangkok, and informed consent was obtained from each participant.

Direct LDL-C assay

Direct LDL-C was determined by selective micellary solubilization of LDL-C by a non-ionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for total cholesterol (TC) determination (cholesterol esterase-cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increased in order as follows: HDL<chylomicrons<VLDL<LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent permits selective determination of LDL-C in serum (Cohn *et al*, 1988; Rifai *et al*, 1992).

Total cholesterol (TC), triglyceride and HDL-C measurement. Total cholesterol and triglycerides levels were measured enzymatically with the CHOD-PAP (Roche Diagnostics, Germany) and lipase/GPO/PAP (Roche Diagnostics, Germany) methods, respectively on a Hitachi 717 analyzer. HDL-C was sequentially determined using polyethyleneglycol (PEG) modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with reactivity increasing in order as follows: LDL<VLDL≈chylomicrons <HDL (Pisani *et al*, 1995; Turkalp *et al*, 2005).

Friedewald calculation. LDL-C was estimated by FF as follows: LDL-C = TC- (HDL-C) - (TG/5) (Friedewald *et al*, 1972).

Statistical analysis

Statistical analysis of the data was performed using the statistical package MINITAB (Ryan *et al*, 1985). Descriptive statistics were used to show the levels of lipid profiles obtained by direct measurement and calculated by FF. The significance level adopted was $p<0.05$. Mean, standard deviation and coefficient of variation were calculated. Correlation of LDL-C by direct method and FF was assessed using the Pearson correlation coefficient.

RESULTS

The levels of serum TC and lipoprotein related to different serum TG levels in this study are shown in Table 1. The levels of se-

Table 1
Summary of the measurements of TC, LDL-C (direct measurement), LDL-C (calculation), and HDL-C according to TG levels, presented as mean ± standard deviation with range.

TG	TC	LDL-C (direct measurement)	LDL-C (calculation)	HDL-C
≤ 150 mg/dl	197.5 ± 46.5	123.7 ± 39.8	119.3 ± 42.0	59.2 ± 19.4
(n = 641)	(70.0-366.0)	(26.0-282.0)	(-11.4-307.6)	(10.0-235.0)
151-200 mg/dl	216.3 ± 48.7	140.1 ± 46.6	130.2 ± 45.7	51.0 ± 15.0
(n = 169)	(100.0-403.0)	(20.0-308.0)	(45.0-312.2)	(5.0-99.0)
201-300 mg/dl	234.7 ± 62.5	155.3 ± 58.0	139.3 ± 62.5	46.8 ± 11.1
(n = 123)	(130.0-541.0)	(47.0-450.0)	(39.4-421.2)	(13.0-85.0)
301-400 mg/dl	238.7 ± 51.5	145.1 ± 50.1	126.6 ± 51.5	42.4 ± 13.7
(n = 41)	(135.0-382.0)	(40.0-272.0)	(54.4-261.4)	(10.0-68.0)
>400 mg/dl	270.2 ± 131.9	144.1 ± 102.5	113.0 ± 106.9	43.4 ± 13.2
(n = 42)	(141.0-992.0)	(17.0-651.0)	(-20.0-624.0)	(14.0-101.0)

TC= total cholesterol, LDL-C= low density lipoprotein cholesterol, TG=triglycerides, HDL-C=high density lipoprotein cholesterol

Table 2
Summary of the measurements of TC, LDL-C (direct measurement), LDL-C (calculation), and HDL-C according to TC levels, presented as mean ± standard deviation with range.

TC	TG	LDL-C (direct measurement)	LDL-C (calculation)	HDL-C
≤ 150 mg/dl	113.4 ± 61.3	71.1 ± 16.3	65.6 ± 17.0	41.5 ± 14.4
(n = 111)	(32.0-554.0)	(26.0-107.0)	(2.2-106.0)	(5.0-80.0)
151-200 mg/dl	129.8 ± 80.2	105.3 ± 18.2	97.4 ± 20.2	54.2 ± 16.6
(n = 353)	(30.0-749.0)	(25.0-154.0)	(-18.8-145.2)	(10.0-165.0)
201-250 mg/dl	162.8 ± 117.7	141.6 ± 22.9	132.1 ± 25.7	57.5 ± 17.6
(n = 354)	(31.0-1,011.0)	(17.0-206.0)	(-20.0-194.4)	(15.0-135.0)
>250 mg/dl	213.6 ± 163.6	196.3 ± 55.7	187.7 ± 56.7	59.9 ± 21.0
(n = 198)	(43.0-1,335.0)	(50.0-651.0)	(47.2-624.0)	(14.0-235.0)

TC= total cholesterol, LDL-C= low density lipoprotein cholesterol, TG=triglycerides, HDL-C=high density lipoprotein cholesterol

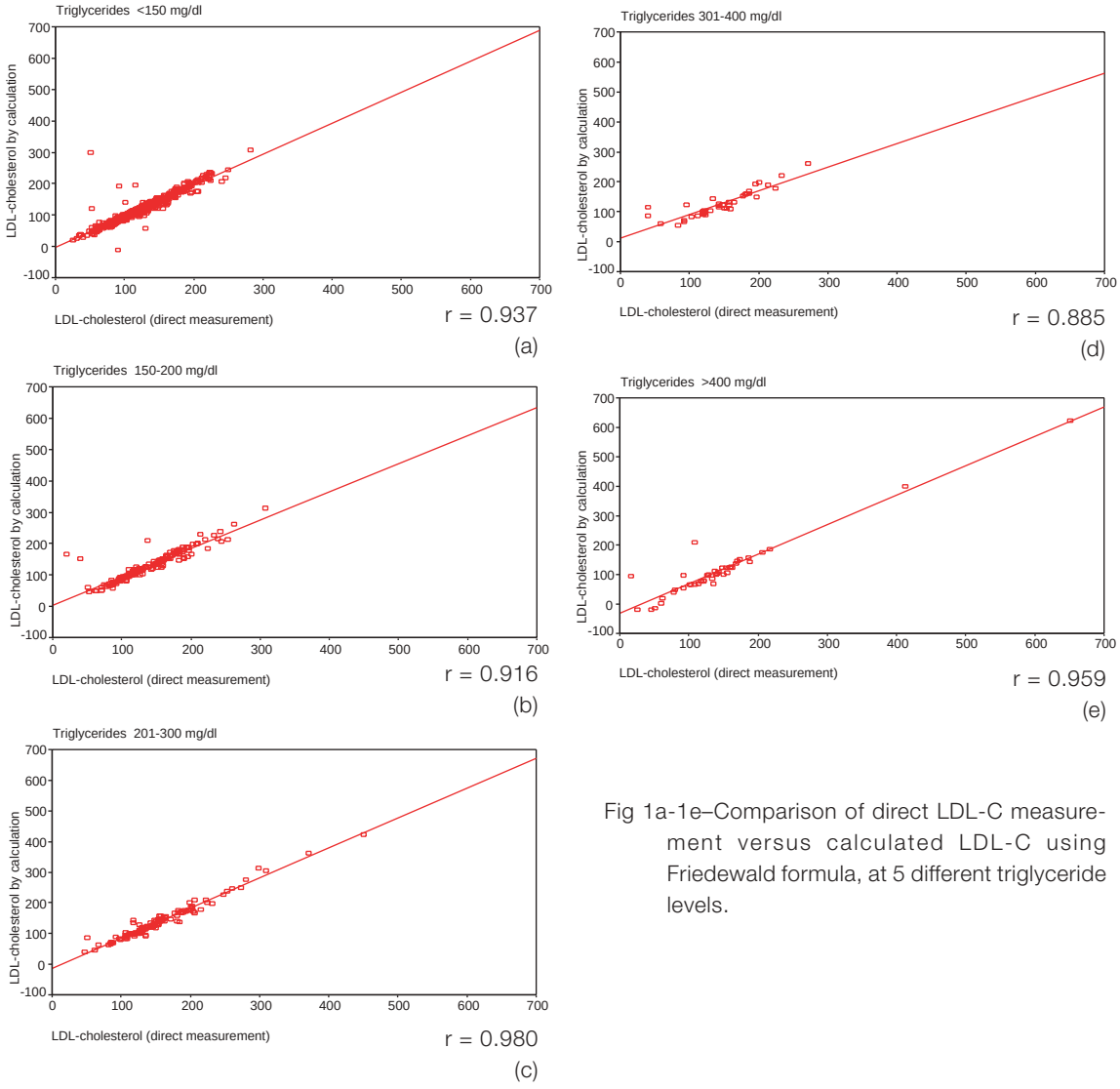

Fig 1a-1e–Comparison of direct LDL-C measurement versus calculated LDL-C using Friedewald formula, at 5 different triglyceride levels.

rum TG and lipoprotein related to different serum TC levels are shown in Table 2.

The correlations between the two methods [direct measurement (x) versus calculated LDL-C (y)] according to the different levels of serum TG are shown in Fig 1a-1e. The highest correlation coefficient (r = 0.980) was seen for TG levels of 201-300 mg/dl. Fig 2a-2d shows the correlation between the methods according to the different levels of serum TC. The maximum correlation coefficient was seen for TC levels of more than 250 mg/dl, with r = 0.881 ($p < 0.05$).

We used multiple regression analysis to predict the level of serum LDL-C from other serum lipid variables: TC, TG, and HDL-C (Table 3). The estimated serum LDL-C level can be calculated from the following equation: LDL-C = 0.910 TC-0.111 TG - 0.634 HDL-C - 6.755 for all ranges of TC, TG, and HDL-C. However, with a serum TG level 201-300 mg/dl which has a maximum correlation coefficient with serum LDL-C level of r = 0.980 the equation changed to:

LDL-C = 0.950 TC-0.088 TG - 0.415 HDL-C -27.016.

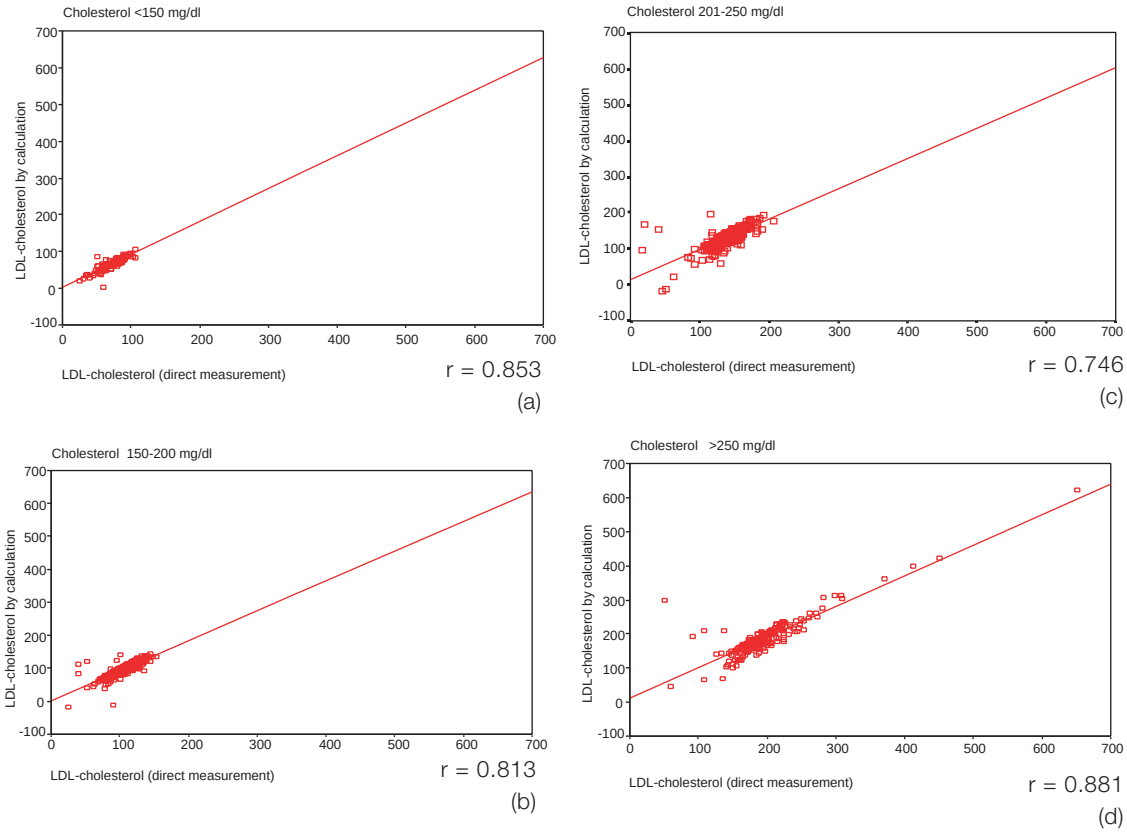

Fig 2a-2d–Comparison of direct LDL-C measurement versus calculated LDL-C using Friedewald formula, at 4 different TC levels.

Using a serum TC level of 250 mg/dl which has a maximum correlation coefficient with a serum LDL-C level of r = 0.881, the equation changed to:

LDL-C = 0.858 TC - 0.102 TG - 0.635 HDL-C - 7.366.

Comparison among the LDL-C measurement methods, homogeneity, and estimation through the Friedewald formula, was performed using regression analysis expressed by the equation $y = bx + a$, where b is the gradient of the line (representing the proportional error) and a is the intersection in the y axis (representing the constant error) (Table 4). The regression equations were as follow: y = 0.988 x -2.840, for TG levels ≤ 150 mg/dl; y = 0.899 x + 4.350 for TG levels of 151-200 mg/dl; y = 0.979 x - 12.780, for TG levels of 201-300 mg/dl ; y = 0.787 x + 12.790, for TG levels of 301-400 mg/dl; and y = 0.959 x - 31.080, for TG levels >400 mg/dl. For the different TG levels, the correlation coefficients 0.937, 0.916, 0.980, 0.885, and 0.959, between the two methods were obtained, respectively. All these correlation coefficients were statistically significant ($p < 0.0001$) (Fig 1a-1e).

DISCUSSION

Our study aimed to assess the performance of a homogeneous method for direct LDL-C measurement, compared with LDL-C estimation using FF. The critical component for assessment of the risk of developing CAD

Table 3
Multivariate analysis of the association of serum LDL-C and TC, TG and HDL-C.

Model		Coefficients[a]			t	Sig
		Unstandardized coefficients		Standardized coefficients		
		B	Std error	Beta		
1	(Constant)	-6.755	2.111		-3.200	0.001
	TC	.910	.010	1.077	93.891	0.000
	TG	-.111	.005	-.264	-22.703	0.000
	HDL-C	-.634	.030	-.237	-21.437	0.000

[a]Dependent variable: LDL-cholesterol (direct measurement)
TC= total cholesterol, LDL-C= low density lipoprotein cholesterol, TG=triglycerides, HDL-C=high density lipoprotein cholesterol

Table 4
Summary of the comparison of LDL-C measurement by direct method and estimation using Friedewald formula, according to TG and TC levels, analyzed using linear regression.

	N	Pearson correlation coefficient	Gradient (95% CI)	Y intersection (95%CI) mg/dl	S_{xy} mg/dl
TG					
≤ 150 mg/dl	641	0.937	0.988 (0.959-1.016)	-2.842 (-6.549-0.864)	14.6
151-200 mg/dl	169	0.916	0.899 (0.838-0.959)	4.349 (-4.545-13.242)	18.3
201-300 mg/dl	123	0.980	0.979 (0.944-1.015)	-12.780 (-18.687-(-6.872))	11.4
301-400 mg/dl	41	0.885	0.787 (0.651-0.922)	12.799(-7.994-33.591)	20.9
>400 mg/dl	42	0.959	1.000 (0.905-1.094)	-31.078 (-47.714-(-14.442))	30.3
TC					
≤ 150 mg/dl	111	0.853	0.853 (0.790-0.997)	2.074 (-5.499-9.647)	8.9
151-200 mg/dl	353	0.813	0.905 (0.837-0.973)	2.157 (-5112-9.427)	11.8
201-250 mg/dl	354	0.746	0.836 (0.758-0.915)	13.697 (2.455-24.939)	17.2
>250 mg/dl	198	0.881	0.896 (0.828-0.963)	11.737 (-2.105-25.580)	26.7

In the form of y=ax+b, while y=calculated LDL-C (Friedewald); x = directly-measured LDL-C; a = gradient of the line; b = y intersection; CI = confidence interval ; S_{xy} =Standard deviation of the residues y.x
TC= total cholesterol, LDL-C= low density lipoprotein cholesterol, TG=triglycerides, HDL-C=high density lipoprotein cholesterol

usually depends on an accurate determination of serum LDL-C concentrations. At present, most clinical biochemical laboratories use FF to determine the serum LDL-C levels, since β-quantification by ultracentrifugation, the reference method to directly measure serum LDL-C, requires considerable time and expense and is not suitable for routine analysis. However, the accuracy of FF has been repeatedly questioned, particularly because it is based on the assumption that the majority of serum TG resides in the VLDL fraction, and the amount of VLDL-C can be estimated by dividing fasting serum TG concen-

trations by a factor of 2.2. Calculated LDL-C is comparable to directly measured LDL-C when TG levels were less than 300 mg/dl. However if serum TG levels were 300 mg/dl or higher, the results of the two methods were significantly different (Table 1). If the results were assessed according to different serum TC levels, the pattern was more acceptable (Table 2).

Although correlation between LDL-C measured by the direct method and estimated by FF has been reported earlier (Saeed *et al*, 2002; Amayo and Kirera, 2004; Cordova *et al*, 2004; Lindsey *et al*, 2004; Turkalp *et al*, 2005), the results of these publications have been arbitrary. Some authors reported lower serum LDL-C β-quantitation after ultracentrifugation (Rifai *et al*, 1998; Nauck *et al*, 2000). One reported bias with a specific different reagent, and showed some correlation with another reagent, although both used detergents, but with different principles of measurement (Esteban-Salan *et al*, 2000). Therefore, we collected a large number of blood samples from Thai patients and compared the LDL-C levels obtained by direct method and calculation by FF. Our study used a reagent with the principle of detergent protection, as used in previous studies (Cohn *et al*, 1988; Rifai *et al*, 1992), which reported no bias when compared with the reference method. Some articles have reported no variation in bias in regard to different levels of TG with a method using specific tensoactive agents (Nauck *et al*, 2000), which is different from most direct methods (Assmann *et al*, 1984; Demacker *et al*, 1984; Mulder *et al*, 1984; Sheikh and Miller, 1985; Yu *et al*, 1997), This may be due to differences in the components of the reagents. In our study, for the comparison of LDL-C measurement by direct method and calculation by FF, for TG levels of 151-200 mg/dl, bias decreased to 18.3 mg/dl, and for TG levels of 201-300 mg/dl, bias almost ceased to exist at 11.4 mg/dl. The bias found in the current experiment seems to be similar to a study by Cordova *et al* (2004) who reported bias in comparison of the direct method of LDL-C measurement with estimate using the FF: for triglyceride levels of 151-200 mg/dl, bias decreased to 14.4 mg/dl, and for levels of 201-300 mg/dl, bias almost disappeared at 15.3 mg/dl. The bias for TG levels of 301-400 mg/dl was 20.9 mg/dl in the current study. This result showed that patients with TG levels of 301-400 mg/dl, and LDL-C levels measured through the direct method of 140 mg/dl, could theoretically have LDL-C levels estimated through the FF of 119.1 mg/dl. The same result would be for patients with LDL-C levels of 170 mg/dl measured through the direct method, which moved from the "high" to "borderline" ranges (149.1 mg/dl). Therefore, the bias of the calculation should be noted when TG levels exceed 300 mg/dl. In conclusion, the direct measurement of serum LDL-C levels has more advantages than the currently used FF as follows: 1) it is easily automated and rapid, 2) both imprecision and bias meet the NCEP performance targets, 3) it has good analytical performance characteristics, 4) it gives reliable results with hypertriglyceridemia, and 5) LDL-C is measured directly, not by estimation from other parameters, therefore the analytical and biological variance can be reduced.

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