Evaluation of Calculated Low-Density Lipoprotein Against a Direct Assay

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Objectives: *To evalate compare the calculated LDL determined by the Friedewald formula when Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL against a direct method.*
Material and Method: *Samples from 202 participants (122 males, 80 females, aged 20-87 years old) were determined for cholesterol, triglyceride (Tg), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) at Department of Laboratory Medicine, King Chulalongkorn Memorial Hospital (KCMH). LDL was determined by Friedewald formula and a direct method.*
Results: *Intra-assay and inter-assay precisions at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL of calculated LDL and direct LDL were 4.80%, 3.29%, 20.37%, 4.86, 8.42%, 8.32%, 2.11%, 1.79%, 3.99%, 2.36%, 2.41% and 6.16%, respectively. The mean absolute biases calculated for calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples were 4.70%, 11.73%, 63.65%, and 7.46%, respectively. Linear regression analysis for calculated LDL vs direct LDL for total samples and grouped as Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL were 0.9190, 0.9796, 0.9440, and 0.7910, respectively. Intraclass correlation coefficient (ICC) at 95% confidence interval of calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples were 0.963, 0.930, 0.767, and 0.889, respectively.*
Conclusion: *The present data suggested that direct LDL is superior over calculated LDL in terms of precision and accuracy. The present study supported that at Tg ≥ 400 mg/dL calculated LDL should not be used and the traditional cutoff of Tg < 400 mg/dL for using Friedewald formula should be revised. In addition, regarding patient convenience, financial reason, and precision and accuracy of analytical method, direct LDL is recommended when Tg ≥ 200 mg/dL.*

Keywords: *Friedewald formula, Calculated LDL, Direct LDL*

J Med Assoc Thai 2005; 88(Suppl 4): S274-9
Full text. e-Journal: *http://www.medassocthai.org/journal*

Nowadays, there is accumulating evidence that reduction of plasma low-density lipoprotein (LDL) concentrations could provide additional benefit in coronary heart disease (CHD) prevention[1], thus, it is no longer to disagree with its important as the cardiovascular risk reduction. Furthermore there is increasing focus on decreasing of CHD risk by reducing plasma levels of LDL, thus LDL concentrations form the basis for treatment guidelines established for hyperlipidemic patients. There is no denying that the precision and accuracy of LDL analysis are important. However, since LDL is very difficult to isolate and measure, therefore the LDL level is widely calculated using the Friedewald formula[2]. In this formula LDL is derived by subtracting high-density lipoprotein (HDL) plus one fifth of triglycerides from total cholesterol. The advantage of this calculated method is easy, convenient, and low cost. Unfortunately, some limitations have occurred from using the formula in determining LDL. Firstly, the end result is based on the accuracy of the testing for the components used to calculate the formula. Secondly, the formulas is accuracy declines with triglycerides over 200 mg/dL and becomes inaccurate

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if the triglycerides exceed 400 mg/dL[3]. Recently, a new generation of direct methods for LDL estimation have been developed which are capable of full automation and suitable for routine laboratories. Some studies demonstrated satisfied accuracy (bias < 4%) and precision (CV < 4%) of these new direct methods[4]. However, most worldwide laboratories still prefer to use the calculated method due to the cost of the direct method.

The present study was aimed to compare the accuracy and precision of calculated LDL determined by the Friedewald formula when Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL against a new generation of a commercial-accepted direct method[4]. The results of the present study could be used as a support data for making the decision to select the assay method for LDL in service laboratories.

Material and Method

Samples

Clotted blood samples were randomly obtained from 250 participants who attained annual health check-up programs at King Chulalongkorn Memorial Hospital (KCMH), from October to December 2004. They also were interviewed and asked to give their consent. Two hundred and two samples without significant appearance or suspect of hemolysis and icterus from 10 hours-fasted participants (122 males, 80 females, age 20-87 years old) were selected. All samples were analyzed on the day of collection at the Department of Laboratory Medicine, KCMH, under the same lipid profile composed of cholesterol, triglyceride (Tg), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). LDL was determined by calculation using Friedewald formula and a direct method using a reagent kit.

Reagents

Direct LDL assay was performed by homogenous enzymatic colorimetric assay, LDL-C plus (second generation, Roche Diagnostics, Cat. No.03038866, System-ID 0766275, Lot No.65891601) on Cobas Integra 400. The reagent was claimed to meet the 1995 the National Cholesterol Education Program (NCEP) goals of precision (CV < 4%) and accuracy (bias ≤ 4%)[5,6]. Intra-assay and inter-assay are 1.1-1.5% and 1.8-1.9%, respectively[7].

Precision studies

Intra-assay precision of calculated LDL and direct LDL were performed using three serum samples with Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL. Each sample was assayed 20 times with the same reagent lot. For the inter-assays, another set of three samples with Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL were assayed in duplicate for 5 days. The mean absolute biases were also calculated for calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples.

Comparative studies

Linear regression analysis was performed for calculated LDL vs direct LDL for a total of 202 samples and grouped as Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL. The correlation (r) of both methods was also determined by comparison studies of the results of the total 202 samples and grouped as Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL.

Statistical analysis

Results were presented as mean ($\overline{X}$) and standard deviation (SD). Differences were examined by the Student's t test. Statistically significant differences were set at $p \leq 0.05$. The association between variables was measured by correlation (r) and linear regression analysis. The mean absolute bias $[\Sigma (| X_i - \overline{X} |) / n]$ was calculated for calculated LDL and direct LDL. The intraclass correlation coefficient (ICC) was used in evaluating the performance of calculated LDL against direct LDL.

Results

Precision studies

Intra-assay and inter-assay precisions of calculated LDL and direct LDL are demonstrated in Table 1. Because the formula's accuracy declines with triglycerides over 200 mg/dL and becomes inaccurate if the Tg exceed 400 mg/dL as stated above[3], the data were grouped by using Tg criteria at < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL. The mean absolute biases were calculated for calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples, and they were 4.70%, 11.73%, 63.65%, and 7.46%, respectively.

Comparison of calculated LDL vs direct LDL

The characteristics of 202 specimens and evaluation of calculated LDL compared with direct LDL according to Tg criteria, < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL are demonstrated in Table 2. Linear regression analysis was performed for calculated LDL vs direct LDL for total 202 samples and grouped as Tg

Table 1. Precision of calculated LDL and direct LDL were performed using three serum samples with Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL

Precision assays	Calculated LDL		Direct LDL	
	Mean ± SD (mg/dL)	CV (%)	Mean ± SD (mg/dL)	CV (%)
Intra-assay (n = 20)				
☐ Tg <200 mg/dL	137.42±6.60	4.80	134.9±2.84	2.11
☐ Tg 200-399 mg/dL	205.16±6.76	3.29	205.1±3.67	1.79
☐ Tg ≥400 mg/dL	34.77±7.08	20.37	55.4±2.21	3.99
Inter-assay (n = 10)				
☐ Tg <200 mg/dL	220.42±10.71	4.86	206.5±4.88	2.36
☐ Tg 200-399 mg/dL	206.16±8.42	8.42	217.2±5.24	2.41
☐ Tg ≥400 mg/dL	84.78±8.32	8.32	94.3±5.81	6.16

Table 2. Evaluation of calculated LDL compare with direct LDL according to triglyceride (Tg) criteria, <200 mg/dL, 200-399 mg/dL, and ≥400 mg/dL

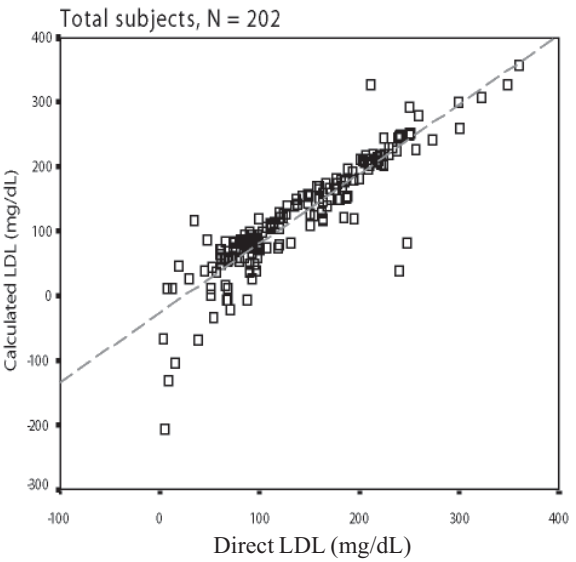
Characteristics	Total	Tg < 200 mg/dL	Tg 200-399 mg/dL	Tg ≥400 mg/dL
Number	202	109	30	63
sex: male/female	122/80	60/49	14/16	48/15
age: X ± SD	49.77±13.85	49.67±14.07	49.1±13.36	50.25±13.91
(range)	(20-87)	(20-87)	(23-80)	(26-79)
Total cholesterol				
mean ± SD	230.98±78.83	216.65±79.56	263.63±61.52	240.21±71.14
minimum	4.00	80.00	117.00	4.00
maximum	466.00	443.00	466.00	390.00
Triglyceride				
mean ± SD	322.15±304.06	120.07±42.43	284.60±61.52	689.65±291.73
minimum	37.00	37.00	210.00	400.00
maximum	1602.00	195.00	396.00	1602.00
HDL				
mean ± SD	47.02±16.93	52.91±17.29	48.60±13.99	36.10±11.55
minimum	5.00	10.00	29.00	5.00
maximum	99.00	99.00	87.00	72.00
Calculated LDL				
mean ± SD	118.40±84.83	138.85±71.34	158.11±79.40	62.39±83.74
minimum	-(207.60)	25.20	-(0.40)	-(207.60)
maximum	358.40	358.40	326.40	252.80
Direct LDL				
mean ± SD	133.46±71.24	143.11±72.53	161.63±66.55	103.35±61.23
minimum	4.00	19.00	5.00	4.00
maximum	360.00	360.00	301.00	250.00
p value	<0.05*	<0.05*	0.4889	<0.05*
Correlation	0.9190	0.9796	0.9440	0.7910

* p ≤ 0.05, statistical significance

< 200 mg/dL, 200-399 mg/dL, and ≥400 mg/dL as seen in Fig. 1-4. ICC (95% confidence interval) of calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples were 0.963, 0.930, 0.767, and 0.889, respectively.

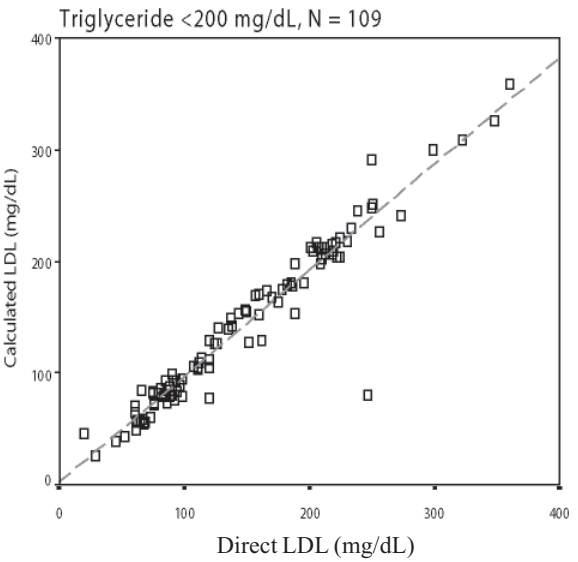
Discussion

LDL is an important key factor in the pathogenesis of premature CHD. Thus, accuracy and precision of assessment of LDL should be considered by all clinical laboratories. The evaluation of validity



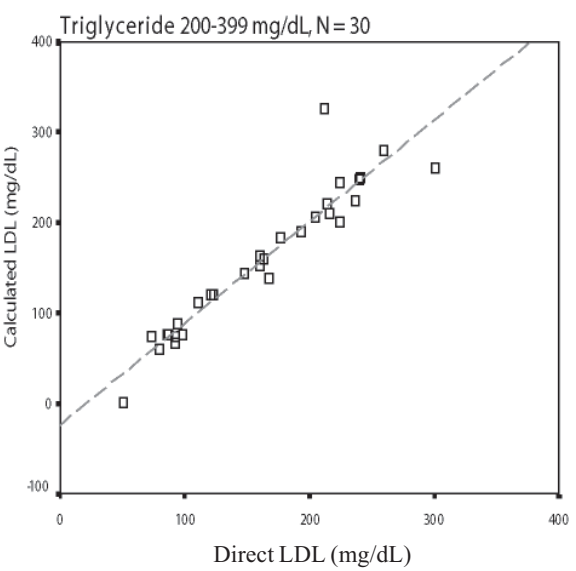
n = 202
r = 0.9190
cal. LDL = 1.077 direct LDL – 26.170

Fig. 1 Linear regression analysis plot of calculated LDL vs direct LDL for total samples (n = 202)



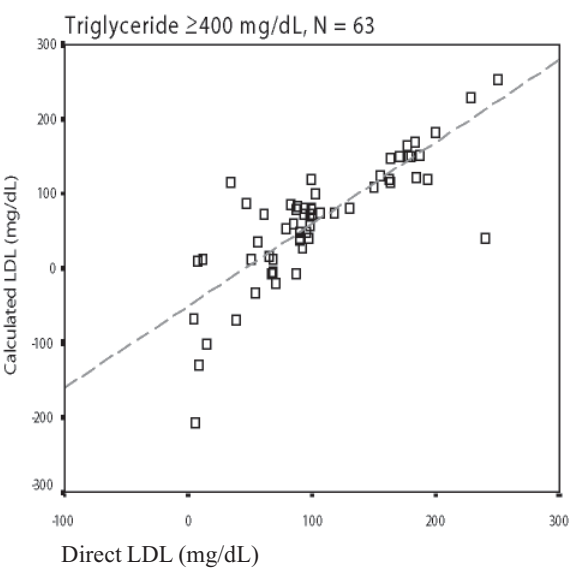
n = 109
r = 0.9796
cal. LDL = 0.950 direct LDL + 2.257

Fig. 2 Linear regression analysis plot of calculated LDL vs direct LDL for Tg < 200 mg/dL (n = 109)



n = 30
r = 0.9440
cal. LDL = 1.126 direct LDL – 23.887

Fig. 3 Linear regression analysis plot of calculated LDL vs direct LDL for Tg 200-399 mg/dL (n = 30)



n = 63
r = 0.7910
cal. LDL = 1.098 direct LDL – 50.639

Fig. 4 Linear regression analysis plot of calculated LDL vs direct LDL for Tg ≥ 400 mg/dL (n = 63)

of worldwide method, calculated LDL using Friedewald formula, against direct LDL should be useful information. Therefore, the authors purpose the present study to compare the calculated LDL against the direct LDL. The comparative study of precision between calculated LDL and direct LDL was performed (Table 1). The authors found that intra-assay and inter-assay precisions of direct LDL were satisfactory (< 4%), but the inter-assay precision at Tg ≥ 400 mg/dL and LDL < 100 mg/dL was less than desirable (6.16%). However, that intra-assay and inter-assay precisions of calculated LDL using Friedewald formula were unacceptable by criteria of NCEP (> 4%), only intra-assay precision at Tg 200-399 mg/dL and LDL > 200 mg/dL was satisfied (3.29%). The presented data suggested that calculated LDL has given unsatisfactory results when Tg ≥ 400 mg/dL and LDL < 100 mg/dL. In addition, the mean absolute biases for calculated LDL against direct LDL at Tg <200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples were 4.70%, 11.73%, 63.65%, and 7.46% compared to direct LDL against the reference method (beta quantification, BQ-LDL) at Tg < 400 mg/dL, and ≥ 400 mg/dL were 12.7% and 30.6%, respectively[8].

Furthermore correlation between calculated LDL and direct LDL was performed and the results at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples were 0.9796, 0.9440, 0.7910, and 0.9190 (Table 2), respectively. The calculated statistic demonstrated different significances at almost all Tg criteria ($p < 0.05$). The authors also calculated ICC (95% confidence interval) to express and compare the reliability index of the test result of the two assays. The ICC for calculated LDL against direct LDL at Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL and for total samples, were 0.963, 0.930, 0.767, and 0.889, respectively. In addition, linear regression studies were studied and demonstrated by Fig. 1-4. Good correlation between calculated LDL and direct LDL was found when Tg < 200 mg/dL (Fig. 2). The higher Tg level, the less desirable correlation between calculated LDL and direct LDL was noticed (Fig. 3 ,4). These results supported that the higher Tg level, the reliability of calculated LDL is lower. The results of the present study were not over the authors' expectation. The reasons for explanation are the Friedewald formula is based on the assumption of a fixed relationship between cholesterol, Tg, and HDL in fasting serum provide that the Tg / cholesterol ratio in VLDL is constant and the Tg is only present as VLDL. Thus, small quantities of chylomicrons and / or chylomicron remnants (e.g. in failure to strictly fasting conditions), or the presence of abnormal lipoproteins inevitably lead to underestimation of LDL[9]. It is also important to remember that the Friedewald formula depends on the accuracy of three different measurements, which can be subject to all the analytical and pre-analytical errors.

Thus, from the present data direct LDL has definitely emerged as a superior assay over calculated LDL in terms of precision and accuracy. In another words, the evidence from the present study suggested that although calculated LDL is cheaper, the accuracy and precision should be cautioned. The present study supported that at Tg ≥ 400 mg/dL calculated LDL should not be used. However, the traditional cutoff of Tg < 400 mg/dL for using Friedewald formula should be revised[3]. In addition, calculation of LDL with Friedewald equation requires patients to fast for 10-12 hours[3], while direct LDL could dispense with fasting samples. In conclusion, regarding convenience to the patients, financial reasons, and precision and accuracy of analytical method, the authors suggested direct LDL should be more suitable performed for samples at Tg ≥ 200 mg/dL. The present also suggested performing direct LDL would be favorable in cases of LDL being ordered without other lipid studies as well as when fasting was doubted.

Acknowledgements

The present study was supported in part by Roche Diagnostics (Thailand) Limited. The authors wish to thank all volunteers, and staff of the Department of Preventive and Social Medicine and Department of Laboratory Medicine, KCMH, for their excellent cooperation, Mr. Wasan Panyasang of the Research Center, for his valuable suggestion in data analysis. The authors also wish to thank Professor Pirom Kamolratanakul, Director of KCMH, and Associated Professor Sophon Napathorn, Deputy Director of KCMH, for commissioning of this study.

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วิธีคำนวณหาค่า low-density lipoprotein เทียบกับวิธีวิเคราะห์โดยตรง

นวพรรณ จารุรักษ์, แอนนา มิลินทากาศ

วัตถุประสงค์: *เพื่อเปรียบเทียบวิธีคำนวณหาค่า low-density lipoprotein ด้วยสูตร Friedewald เมื่อ Tg < 200 mg/dL, 200-399 mg/dL, and ≥ 400 mg/dL เทียบกับวิธีวิเคราะห์โดยตรงวิธีใหม่*

วัสดุและวิธีการ: *ตัวอย่างสิ่งส่งตรวจจากกลุ่มผู้ร่วมการศึกษาจำนวน 202 ราย (ชาย 122 คน และหญิง 80 คน อายุระหว่าง 20-87 ปี) ได้รับการตรวจ cholesterol, triglyceride (Tg), high-density lipoprotein (HDL), และ low-density lipoprotein (LDL) จากฝ่ายเวชศาสตร์ชันสูตร โรงพยาบาลจุฬาลงกรณ์ ค่า LDL หาโดยการคำนวณด้วยสูตร Friedewald และวิธีวิเคราะห์ โดยตรงด้วยน้ำยา*

ผลการศึกษา: *เปรียบเทียบ Intra-assay และ inter-assay เมื่อ Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL ของวิธีคำนวณและวิธีวิเคราะห์โดยตรงสำหรับ LDL เท่ากับ 4.80%, 3.29%, 20.37%, 4.86, 8.42%, 8.32%, 2.11%, 1.79%, 3.99%, 2.36%, 2.41% and 6.16%, ตามลำดับ ค่าเฉลี่ยของค่าอคติสัมบูรณ์สำหรับวิธีคำนวณต่อวิธีวิเคราะห์โดยตรง เมื่อ Tg < 200 mg/dL, 200-399 mg/dL, ≥400 mg/dL และของตัวอย่างทั้งหมด เท่ากับ 4.70%, 11.73%, 63.65%, และ 7.46%, ตามลำดับ ค่าถดถอยเชิงเส้นตรงของวิธีคำนวณต่อวิธีวิเคราะห์โดยตรงของตัวอย่างทั้งหมด และเมื่อ Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL เท่ากับ 0.9190, 0.9796, 0.9440, และ 0.7910, ตามลำดับ ค่า Intraclass correlation coefficient (ICC) ที่ความเชื่อมั่น 95 % ของวิธีคำนวณต่อวิธีวิเคราะห์โดยตรง เมื่อ Tg < 200 mg/dL, 200-399 mg/dL, ≥ 400 mg/dL และของตัวอย่างทั้งหมด เท่ากับ 0.963, 0.930, 0.767, และ 0.889, ตามลำดับ*

สรุป: *ผลการศึกษานี้พบว่าวิธีวิเคราะห์ LDL โดยตรงเหนือกว่าวิธีคำนวณทั้งในด้านความแม่นยำและความถูกต้อง การศึกษานี้ ยังสนับสนุนว่าไม่ควรใช้วิธีคำนวณ เมื่อ Tg ≥ 400 mg/dL และวิธีคำนวณด้วยสูตร Friedewald เมื่อ Tg ≥ 400 mg/dL ควรได้รับ การทบทวน นอกจากนี้เพื่อความสะดวกค่าใช้จ่ายความแม่นยำและความถูกต้อง การใช้วิธีวิเคราะห์โดยตรง ควรใช้เมื่อ Tg ≥ 200 mg/dL*