Analytical and Clinical Performance of Two Homogeneous Assays for Measuring of LDL-Cholesterol

WATTANA LEOWATTANA, M.D.*, SOMJAI NARKRUNG, B.Sc.*,
SASIKANT POKUM, B.Sc.*, SUDCHAREE KIARTIVICH, M.Sc.*

Abstract

A clinical laboratory currently estimates LDL-Cholesterol (LDL-C) concentration using the Friedewald calculation, which requires fasting specimens and is subject to error with increasing triglycerides levels. We evaluated the analytical and clinical performance of the direct LDL-C assay from two companies, Roche Diagnostics ($LDL\text{-}C_{Roche}$) and Wako Pure Chemical ($LDL\text{-}C_{Wako}$). Both methods meet current guidelines for precision with within-run coefficients of variation less than 3 per cent. The $LDL\text{-}C_{Roche}$ assay correlated well with the LDL-C from the Friedewald equation ($LDL\text{-}C_{Fried}$, $r = 0.958$, $y = 0.85x + 17.08$ mg/dL, $n = 422$). The $LDL\text{-}C_{Wako}$ assay also correlated with the $LDL\text{-}C_{Fried}$ ($r = 0.946$, $y = 0.86x + 7.81$ mg/dL, $n = 422$). In addition, at the medical decision cutoff points, $LDL\text{-}C_{Roche}$ assay and $LDL\text{-}C_{Wako}$ showed positive predictive values of 87.44 per cent and 69.67 per cent respectively. We conclude that the $LDL\text{-}C_{Roche}$ assay meets the currently established analytical and clinical performance, but $LDL\text{-}C_{Wako}$ assay meets only analytical performance. Clinical performance needs further evaluation.

Key word : Homogeneous LDL-C Assays, Analytical and Clinical Performance

LEOWATTANA W, NARKRUNG S, POKUM S, KIARTIVICH S
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The association of total cholesterol (TC) and LDL-Cholesterol (LDL-C) concentrations with risk of coronary artery disease (CAD) is well-established[1-3]. In humans, LDL carries most of the circulating cholesterol. It is necessary in the diagnosis and treatment of hyperlipidemia, therefore, it is important to establish the reliable measurement of LDL-C. Moreover, according to the National Cholesterol Education Program-Adult Treatment Panel II (NCEP-ATP II) recommenda-

* Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

tions, the diagnosis and treatment of adult patients with hypercholesterolemia are based on LDL-C concentration. The β-quantification method, which involves an ultracentrifugation step, is a generally accepted method to determine LDL-C. However, this method is confined only to the research laboratory because the technique is labor intensive and its throughput is limited. In addition, the Friedewald calculation ($LDL\text{-}C_{Fried}$) is the most commonly used procedure in the clinical laboratories for the estimation of LDL-C. Although $LDL\text{-}C_{Fried}$ correlates highly with the β-quantification method, it has several drawbacks. It is invalid when a specimen is collected in the nonfasting state or from the patients with type III hyperlipoproteinemia or in the presence of triglycerides (TG) more than 400 mg/dL. Therefore, the NCEP Working Group on Lipoprotein Measurement recommended the development of direct methods for LDL-C measurement. Chemical precipitation and immunoprecipitation methods for the quantification of LDL-C concentration have been reported (4-6). These methods are affected by TG concentration, do not measure all LDL components and require a pretreatment step. Here we describe the analytical and clinical performance of two types of direct homogeneous LDL-C assays. We also compare these methods with the Friedewald calculation.

MATERIAL AND METHOD

Samples

Fasting serum from 471 patients with wide range of TC and TG concentration were obtained. All of the samples were analyzed for TC, TG and HDL-Cholesterol (HDL-C) to calculate $LDL\text{-}C_{Fried}$. Homogeneous LDL-C assay was performed by 2 methods, the first one was a detergent-based homogeneous assay, and the other one was a polyanion and amphoteric surfactant protective assay.

Lipid measurements

TC and TG were determined enzymatically on the Hitachi 917 analyzer (Roche Diagnostics, Thailand). The day-to-day imprecision of the two methods, reflected by the CV when Precinorm® and Precipath® controls were used, was less than 3 per cent. HDL-C was measured by using a homogeneous assay (Roche Diagnostics, Thailand) with a day-to-day CV less than 3 per cent.

$LDL\text{-}C_{Roche}$ assay

At neutral pH (pH 7.0) and in the presence of the $MgCl_2$, sulfated α-cyclodextrin and dextran sulfate, the enzymatic reaction for cholesterol in VLDL and chylomicrons is markedly reduced reagent 1. The nonionic detergent in reagent 2, which selectively solubilizes LDL-C but not HDL-C, enables the measurement of LDL-C by a conventional enzymatic reaction. The assay was calculated as recommended with the Calibrator for automated system (C.f.a.s) LDL-C Plus calibrator, and performed according to the manufacturer' s recommendation.

$LDL\text{-}C_{Wako}$ assay

This assay was performed according to the manufacturer's specifications on the Hitachi 917 analyzer. The assay is available from Wako Pure Chemical, Japan. At pH 6.8 and containing N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS), polyanion and amphoteric surfactant protect LDL from enzyme reactions. Cholesterol esterase (CE) and cholesterol oxidase (CO) react with non-LDL lipoproteins (chylomicrons, VLDL and HDL). Hydrogen peroxide produced by the enzyme reaction with non-LDL cholesterol is decomposed to water by catalase in reagent 1. When regent 2 is added, CE and CO reacts only with LDL-C. Hydrogen peroxide produced by the enzyme reactions with LDL-C yields a color complex upon oxidative condensation with HDAOS and 4-aminoantipyrine (4AA). By measuring the absorbance of the blue color complex produced at 600 nm, the LDL-C concentration in the sample can be calculated compared with the absorbance of the LDL-C calibrator.

$LDL\text{-}C_{Fried}$ calculation

$LDL\text{-}C_{Fried}$ was estimated by the Friedewald calculation [LDL-C = TC – (HDL-C + TG /5)] only when fasting TG are less than 400 mg/dL, where TG/5 is an estimate of VLDL-C, and all concentrations are expressed in mg/dL.

Statistical analysis

The means and standard deviation were calculated with Microsoft Excel, Ver. 5.0 (Microsoft). Student's *t*-test and least-squares linear regression analysis were performed using StatView 4.51 software. The *t*-test was considered significant at $p < 0.05$. The positive predictive value (PPV) of

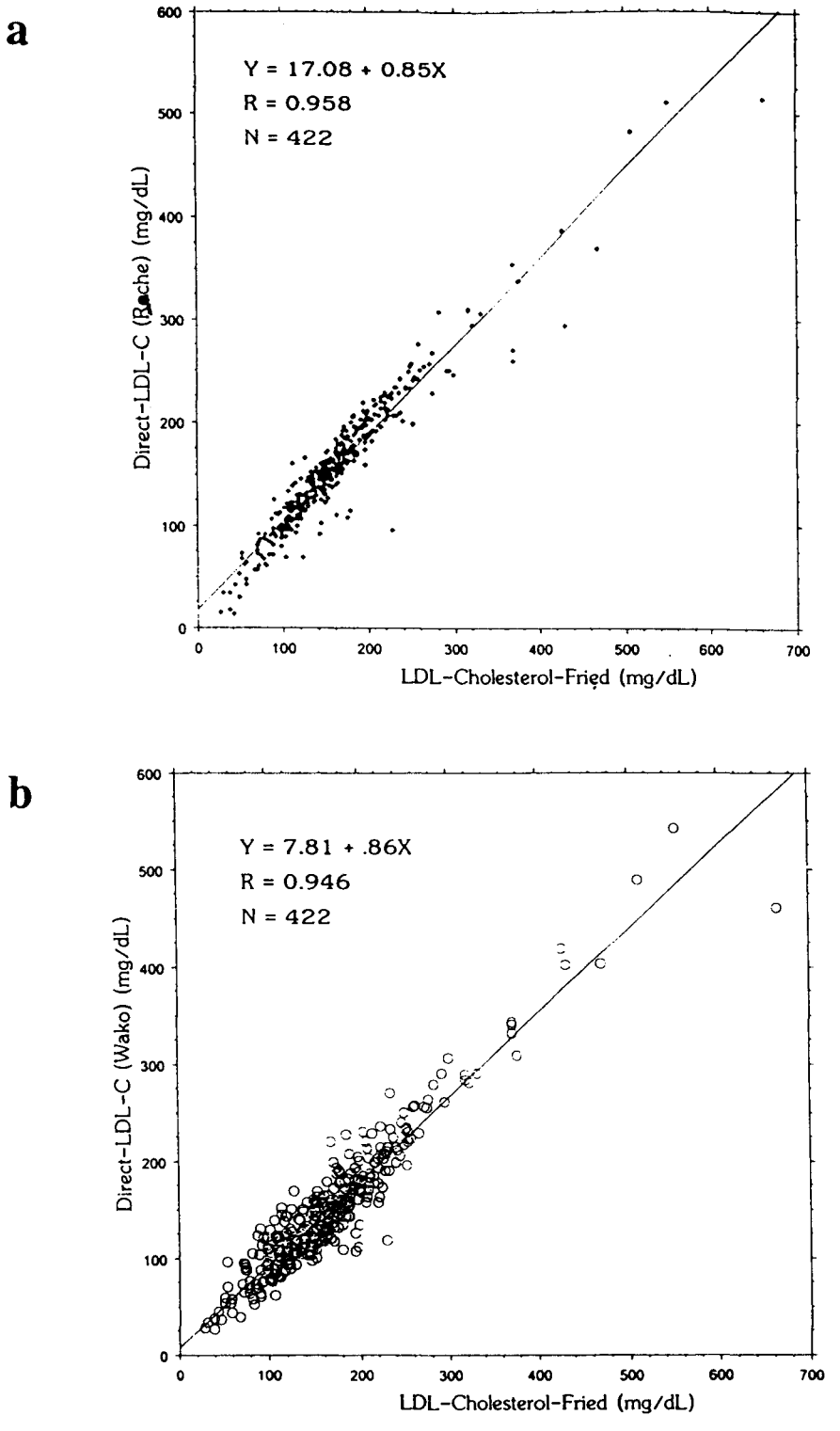

Fig. 1. Linear regression analysis plot of LDL-C_{Roche} vs LDL-C_{Fried} (a) and LDL-C_{Wako} vs. LDL-C_{Fried} (b) for triglycerides concentration less than 400 mg/dL.

an LDL-C assay was calculated as [true positive/(true positive + false positive)] x 100. True positive meant that LDL-C results of both the reference method (LDL-C $_{Fried}$) and the test methods were greater than or equal to the cutoff concentration. False positive meant that the test method LDL-C result was greater than the cutoff point when the reference procedure LDL-C value was less than the cutoff point.

RESULTS

Precision studies

The precision profile for LDL-C $_{Roche}$ and LDL-C $_{Wako}$ assay was performed with normal, borderline, and high concentrations of LDL-C. The intraassay CV of LDL-C $_{Roche}$ and LDL-C $_{Wako}$ assay for all concentrations of LDL-C were less than 3 per cent (Table 1).

Table 1. Precision profile for the LDL-C$_{Roche}$ and LDL-C$_{Wako}$ assay.

	Intraassay (n = 20)			
	LDL-C$_{Roche}$ Mean ± SD mg/dL	CV %	LDL-C$_{Wako}$ Mean ± SD mg/dL	CV %
Level 1	62.63±1.19	1.9	59.40±0.84	1.4
Level 2	108.83±1.72	1.6	102.25±0.96	0.9
Level 3	195.80±4.02	2.1	199.20±5.55	2.8

Method Comparison

Linear regression analysis was performed for LDL-C $_{Roche}$ and LDL-C $_{Wako}$ vs LDL-C $_{Fried}$ for TG concentrations less than 400 mg/dL. As seen in Fig 1a and 1b, both LDL-C$_{Roche}$ and LDL-C$_{Wako}$ show a good correlation (r = 0.958

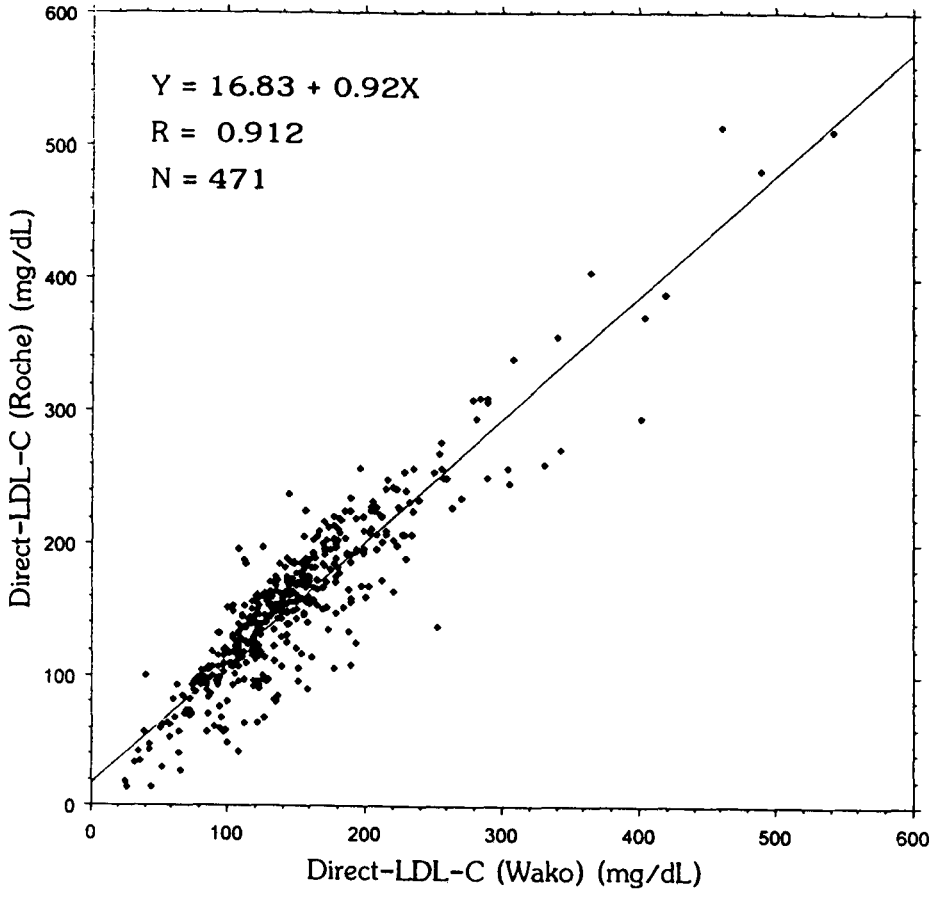

Fig. 2. Linear regression analysis plot of LDL-C$_{Roche}$ vs. LDL-C$_{Wako}$ for all range of triglycerides concentration.

Table 2. LDL-C measured directly by LDL-C_{Roche} assay, LDL-C_{Wako} assay and calculated by the Friedewald equation (LDL-C_{Fried}).

N	All 471	TG<130 mg/dL 191	130<TG<400 mg/dL 238	TG>400 mg/dL 42
LDL-C_{Roche}	153.07±63.85	145.97±51.34	164.90±70.05	123.04±64.31
LDL-C_{Wako}	148.09±63.29	130.47±50.05	162.19±71.15	148.98±49.64
LDL-C_{Fried}	162.22±75.02	153.63±57.75	174.63±86.24	122.42±52.87

a

LDL-C_{Fried} \ LDL-C_{Roche}	< 130	130 - 160	> 160
< 130	93.23% 124/133	6.02% 8/133	0.75% 1/133
130 - 160	12.37% 12/97	78.35% 76/97	9.28% 9/97
> 160	1.56% 3/192	10.42% 20/192	88.02% 169/192

b

LDL-C_{Fried} \ LDL-C_{Wako}	< 130	130 - 160	> 160
< 130	92.48% 123/133	6.77% 9/133	0.75% 1/133
130 - 160	50.52% 49/97	44.33% 43/97	5.15% 5/97
> 160	3.65% 7/192	29.68% 57/192	66.67% 128/192

Fig. 3. Diagnostic performance of LDL-C_{Roche} and LDL-C_{Wako} compare with LDL-C_{Fried} (a and b) according to NCEP guidelines.

and 0.946, respectively). Fig. 2 shows the good correlation of LDL-C_{Roche} and LDL-C_{Wako} for the TG concentration range between 39-1,383 mg/dL (r = 0.912). Table 2 shows the mean value of LDL-C obtained by each method for the overall TG range as well as for TG concentrations less than 130 mg/dL, between 130 – 400 mg/dL and more than 400 mg/dL. The mean value of LDL-C_{Roche} was slightly higher than LDL-C_{Wako} for the overall TG range. However, the mean value of LDL-C_{Fried} was higher than both LDL-C_{Roche} and LDL-C_{Wako} for the overall TG range.

Data classification using NCEP guidelines

The NCEP has established LDL-C at points of <130, 130 to 159, and ≥160 mg/dL that classify asymptomatic patients into acceptable, borderline, and high categories, respectively. In the fasting state, the LDL-C_{Roche} assay correctly classified 87.44 per cent of the patients compared with the LDL-C_{Wako} assay, which correctly classified only 69.69 per cent of the patients (Fig. 3a, 3b respectively).

DISCUSSION

LDL-C is a key factor in the pathogenesis of premature coronary artery disease (CAD). The availability of an accurate and precise method to evaluate LDL-C is a very importance factor in the clinical assessment of patients at risk for CAD. In addition, the reduction of increased LDL-C is a major goal for the primary and secondary prevention of coronary heart disease. The Friedewald calculation for estimating the LDL-C concentration is the routine method currently recommended by the NCEP Working Group for Lipoprotein measurement. Because of the drawbacks of this calculation, methods for the direct determinations of LDL-C are needed. Homogeneous methods have the apparent advantage of obviating the need for pretreatment of samples, being performed by automated analyzer, and requiring only a few microliters of sample.

From our study, good agreement was seen between the LDL-C_{Roche}, LDL-C_{Wako} and LDL-C_{Fried} procedures in the case of TG concentration less than 400 mg/dL as reported by other investigators [7-9]. When we measured LDL-C_{Roche} and LDL-C_{Wako} from specimens with TG concentration more than 400 mg/dL, where the Friedewald calculation is unreliable, the correlation was also good although LDL-C_{Wako} seemed to be lower than LDL-C_{Roche}. Furthermore, the results of the method comparison support that the homogeneous LDL-C_{Roche} and LDL-C_{Wako} assay can be used in the determination of LDL-C in hyperlipidemic patients.

According to the NCEP guidelines, the management of hyperlipidemic patients, using either dietary or drug therapy, is based on three LDL-C cut –points (130, 160 or 190 mg/dL). LDL-C concentrations determined by either the LDL-C $_{Roche}$ assay or LDL-C $_{Wako}$ assay correctly classified 87.44 per cent and 69.69 per cent of the subjects, respectively. The LDL-C $_{Roche}$ assay but not the LDL-C $_{Wako}$ assay was able to correctly classify into NCEP cut-points nearly all subjects, except subjects with TG concentrations of more than 400 mg/dL.

The limitation of this study is that the gold standard method for LDL-C assay (β-quantification) was not done in parallel with LDL-C_{Roche} and LDL-C_{Wako}. However, there are many reports that confirmed the correlation between LDL-C_{Roche} assay with β-quantification assay [7,9].

In conclusion, the homogeneous LDL-C_{Roche} assay is precise and acceptably accurate. The LDL-C_{Wako} assay is also precise but the concentration is slightly lower than LDL-C_{Roche} and LDL-C_{Fried}. The positive predictive value of LDL-C_{Roche} is also better than LDL-C_{Wako}. On the basis of the finding of this study the authors would favor the LDL-C_{Roche} assay over LDL-C_{Wako} assay. In hypertriglyceridemic patients (fasting or non-fasting), the evaluation of LDL-C assay is too inaccurate to prove it to be a reliable assay not only LDL-C_{Fried} but also LDL-C_{Roche} and LDL-C_{Wako}. We strongly believe that in patients with such high TG concentrations, instead of spending time and effort determining LDL-C, the prudent clinician would be more concerned in lowering the TG value and with it the attendant immediate risk of pancreatitis before assessing cardiovascular risk.

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การศึกษาเปรียบเทียบประสิทธิภาพในการทดสอบหาระดับแอลดีแอล-โฆเลสเตอรอล โดยวิธีวัดตรงเปรียบเทียบระหว่าง 2 วิธี

วัฒนา เลี้ยววัฒนา, พ.บ., สมใจ นาคหรั่ง, วท.บ.*, ศศิกานต์ โพธิ์คำ, วท.บ.*, สุทธิ์จรี เกียรติวิชญ์, วท.ม.**

โดยปกติห้องปฏิบัติการที่ให้บริการตรวจเลือดทางเคมีคลินิกจะใช้วิธีการคำนวณหาระดับแอลดีแอล-โฆเลสเตอรอลด้วยสูตรของ ฟรีเดอวาล ซึ่งผู้ป่วยจะต้องงดอาหารและน้ำดื่มก่อนการตรวจเลือดและต้องมีระดับไตรกลีเซอไรด์ไม่เกิน 400 มก./ดล. คณะผู้วิจัยได้ทำการศึกษาประสิทธิภาพของการตรวจวัดระดับ แอลดีแอล-โฆเลสเตอรอลโดยวิธีวัดตรง 2 วิธีจาก บริษัทโรช ไดแอกโนสติก และบริษัทวาโก้ เพียวเคมิคอล พบว่าทั้ง 2 วิธีมีความแม่นยำอยู่ในเกณฑ์น้อยกว่า 3 เปอร์เซ็นต์, ความสัมพันธ์ระหว่างแอลดีแอล-โฆเลสเตอรอล-โรช กับ แอลดีแอล-โฆเลสเตอรอล-ฟรีเดอวาล อยู่ในเกณฑ์ดี ($r = 0.958$, $y = 0.85x + 17.08$ mg/dL, $n = 422$) ส่วนความสัมพันธ์ระหว่างแอลดีแอล-โฆเลสเตอรอล-วาโก้ กับ แอลดีแอล-โฆเลสเตอรอล-ฟรีเดอวาลก็อยู่ในเกณฑ์ดีเช่นกัน ($r = 0.946$, $y = 0.86x + 7.81$ mg/dL, $n = 422$). นอกเหนือจากนั้นยังพบว่า แอลดีแอล-โฆเลสเตอรอล-โรช กับ แอลดีแอล-โฆเลสเตอรอล-วาโก้ มี positive predictive value เท่ากับ 87.44 เปอร์เซ็นต์ และ 69.67 เปอร์เซ็นต์ตามลำดับ คณะผู้วิจัยสรุปว่า แอลดีแอล-โฆเลสเตอรอล-โรช เป็นวิธีการตรวจหาแอลดีแอล-โฆเลสเตอรอล แบบตรงได้ดีกว่าแอลดีแอล-โฆเลสเตอรอล-วาโก้.

คำสำคัญ : แอลดีแอล-โฆเลสเตอรอล, การตรวจวัดแบบตรง

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* ภาควิชาพยาธิวิทยาคลินิก, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ 10700