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A Critical Review of LDL Cholesterol and HDL Cholesterol Measurement

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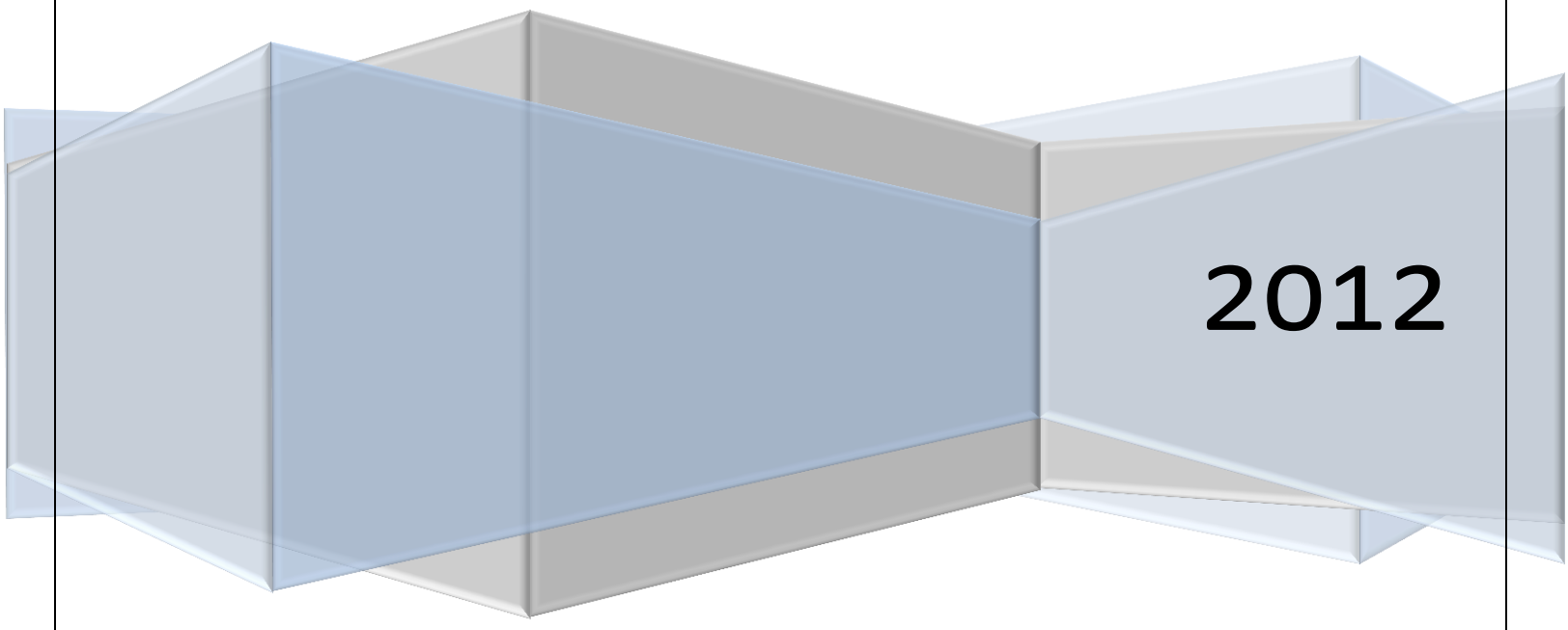


Summary

Measurement of low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol is the cornerstone of cardiovascular disease risk assessment. Yet few of us appreciate the potential for error due to imprecision and inaccuracy. Despite the widespread belief that the calculation or measurement of LDL or HDL cholesterol is standardized and reproducible, the data indicate that results can vary significantly with methods from different manufacturers, and calculated LDL cholesterol may not agree with measured LDL cholesterol. Problems with direct HDL-C assays also raise concerns about the reliability of calculated LDL cholesterol and non-HDL cholesterol measurement. Poor reliability of these assays relate to the ambiguity in the definition of both LDL and HDL particles, and the heterogeneity of LDL and HDL particles (1).

For clinical research organizations (CROs), contract laboratories, and academic and pharmaceutical research laboratories developing next generation lipid therapies and diagnostics, choice of methods is vitally important. Clinical laboratories, especially those that specialize in lipid testing, should also consider assay reliability when choosing methods. This manuscript examines the reliability of current methodologies for measurement of LDL and HDL cholesterol and suggests that "old school" methods, such as dextran sulfate precipitation, perform better than newer homogeneous assays.

Introduction

There is a direct relationship between serum LDL cholesterol and the incidence of coronary heart disease (CHD). Similarly, there is an inverse association between HDL cholesterol and CHD. Intervention with a variety of drugs such as statins, fibrates, bile acid resins, and niacin will decrease LDL cholesterol and subsequent CHD risk. The National Cholesterol Education Program (NCEP) recommends that all adults over the age of 20 be screened for CHD risk with measurement of total cholesterol (TC), LDL cholesterol, HDL cholesterol, and triglyceride (TG) concentrations, while treatment decisions are largely based on LDL cholesterol concentrations (2).

In high risk individuals, the recommended LDL cholesterol therapeutic goal is <100 mg/dL, with an optional goal of <70 mg/dL or a 30-40% reduction in LDL-C levels (3). In clinical laboratories LDL-C is most often determined using the "Friedewald formula" using measured values for total cholesterol, HDL-C, and triglyceride:

LDL-C = (TC) - (HDL-C) - (TG/5); where units are mg/dL

This calculation is not valid for specimens having TG >400 mg/dL, for patients with Type III hyperlipoproteinemia, fasting chylomicronemia, or who are nonfasting. Homogeneous assays for LDL cholesterol measurement are gaining in popularity.

HDL particles are anti-atherogenic; they promote cellular cholesterol efflux and reverse cholesterol transport, and they also appear to have anti-inflammatory, antioxidant, and anticoagulant properties. Epidemiological studies demonstrate an inverse association between HDL-C concentration and coronary heart disease (CHD). According to NCEP guidelines, HDL-C <40 mg/dL is a risk factor for CHD. HDL-C≥60 mg/dL is a "negative" risk factor; its presence subtracts one risk factor from the total count (2).

NCEP has developed programs to encourage standardization of these assays and improve the reliability of testing. The NCEP Working Group on Lipoprotein Measurement (WGL) analytical performance guidelines for TC, TG, LDL-cholesterol, and HDL cholesterol (4-7) are summarized in Table 1.

Table 1: Performance Goals for lipid and lipoprotein testing as defined by the NCEP Laboratory Standardization Panel and the Working Group on Lipoprotein Measurement

	Total error,%	Precision,%	Bias,%
Total Cholesterol	≤9	≤3	≤3
Triglycerides	≤15	≤5	≤5
LDL Cholesterol	≤12	≤4	≤4
HDL Cholesterol	≤13	≤4	≤5

Performance goals are based on total error. Bias and precision estimates are conditions consistent with goals for TE.

Errors in Lipid Measurement

Analytical laboratory error can be divided into two components: imprecision and inaccuracy. Precision refers to the reproducibility of a particular method, often reported as a coefficient of variation (CV). Inaccuracy, or bias, refers to a systematic difference in results between a method and the "true" or reference value. Manufacturers of lipid assays (TC, TG, LDL cholesterol, and HDL cholesterol) standardize their assays by "split sample" comparison with a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory using fresh patient samples to minimize matrix effects. The CRMLN

laboratories are, in turn, traceable to the CDC reference methods. This process ensures that the calibrators and reagents sold by manufacturers are traceable to the CDC reference methods. Although TC standardization is generally viewed as a success, there remain concerns about the effectiveness of standardization programs for other analytes. An important limitation of the standardization protocol is the lack of testing with specimens from individuals with lipoprotein abnormalities to better evaluate "real world" assay performance.

Errors in LDL Cholesterol Measurement

"LDL" and "LDL cholesterol" are often− and incorrectly−used interchangeably. LDL are particles of various sizes containing various proportions of cholesterol, triglycerides, phospholipids, protein, and other lipid soluble substances. LDL cholesterol is the mass of cholesterol within LDL particles.

In clinical practice, LDL-C is either estimated by the Friedewald formula or directly measured with a homogeneous assay. Because the calculation is based on plasma TG, TC, and HDL cholesterol, it necessarily includes the accumulated errors in all three measurements. Calculated LDL cholesterol does not add additional cost beyond the three core measurements, but requires a fasting sample, while direct measurement avoids the need for fasting samples.

LDL has also been defined based on electrophoretic mobility as beta-migrating lipoproteins or as the lipoprotein particles in the density range 1.019 to 1.063 kg/L after ultracentrifugation. However, risk-based cut points for LDL cholesterol defined by NCEP are based on epidemiological studies, almost all of which used either beta-quantification (BQ) or LDL cholesterol calculated using the Friedewald equation, which was derived from BQ.

BQ, the reference method on which standardization of LDL-C is based, requires ultracentrifugation of serum or plasma at d = 1.006 kg/L to separate the supernatant, which contains VLDL and chylomicron, from the infranate, which contains LDL and HDL. Cholesterol is measured in the infranate to provide the sum of LDL and HDL cholesterol, and then LDL particles are precipitated from the infranate, and HDL cholesterol is measured in the remaining supernatant. LDL cholesterol is then calculated as infranate cholesterol minus HDL cholesterol. Thus, BQ LDL cholesterol measurement includes the cholesterol in intermediate-density lipoproteins (IDL; 1.006-1.019 kg/L) and the cholesterol in lipoprotein(a) [Lp(a)]. VLDL remnants can also be found in the intermediate density range (1.006-1.019 kg/L). Although the BQ methodology is the reference method, it is technically demanding and

requires an ultracentrifuge. In order for calculated or directly measured LDL cholesterol to agree with BQ, IDL and Lp(a) cholesterol and cholesterol from LDL of different sizes and composition must be included. Intuitively, it is easy to understand the difficulty in developing direct methods that will provide the necessary accuracy and precision.

The Friedewald Equation

Limitations of the Friedewald equation were recognized early on: the calculation is not valid for specimens having triglycerides >400 mg/dL, for patients with Type III hyperlipoproteinemia or chylomicronemia, or with nonfasting specimens (8). In fact, the equation is increasingly inaccurate with TG from 200 to 400 mg/dL (9-10). In diabetic patients, the Friedewald formula underestimated LDL cholesterol by 8-10% and agreement within 10% of BQ was seen in only 68% of diabetic patients (11). Another report also showed poor correlation between the Friedewald equation and ultracentrifugation with only 49% and 73% of results agreeing within 10% for diabetics and non-diabetics, respectively (12). The differences between methods were apparently related to differences in triglyceride concentrations (13). Scharnagl et al. found that the Friedewald equation was inaccurate at lower LDL-C concentrations compared with BQ (14). A recent study reported poor agreement between results calculated with the Friedewald equation and a direct LDL-C assay from Siemens; despite a good correlation, more than 25% of results differed by more than 30 mg/dL (15).

Direct LDL-C Assays

"Direct" refers to homogeneous methods that do not require a preliminary separation step or manual manipulation of the sample. Given the impracticality of electrophoresis and ultracentrifugation in the clinical laboratory, alternate methods for separating LDL were sought. Genzyme introduced a second generation method involving immunoseparation, using apo AI and apo E antibodies bound to polystyrene beads to bind VLDL and HDL, while LDL were collected in the filtrate. The assay was relatively robust, although some VLDL remained with the LDL fraction in hypertriglyceridemic specimens resulting in a 5% to 12.5% positive bias (16). Also, no IDL and only about 75% of the Lp(a) cholesterol was retained with the LDL fraction (16).

The third generation assays were true homogeneous methods. There are at least six direct LDL-C methods currently available, each based on different proprietary principles (Table 2). Despite the ease of use, numerous reports have questioned the performance of these assays.

Miller et al. compared four direct methods to the BQ reference method with samples from 100 subjects, including 60 with dyslipidemias (17). Total error was 12.6% to 41.6%, with none of the methods meeting the NCEP total error performance goal of < 12% (17).

Not surprisingly, these direct methods differ in recognition of different lipoprotein fractions and subclasses. Fei et al. compared the Kyowa and Daiichi direct LDL-C assays and determined that both methods measured only a fraction of the IDL cholesterol (Kyowa: 52%; Daiichi: 31%) while measuring a proportion of apoE-rich HDL as LDL (Kyowa: 18%; Daiichi: 8%) (18). The Daiichi method also measured abnormal lipoprotein Lp-X, isolated from cholestatic serum as LDL cholesterol (18-19).

Table 2: Direct LDL- Cholesterol Assays

1. Sekisui Medical (formerly Daiichi) liquid selective detergent method:
CM, VLDL, HDL + CE + CO + surfactant 1 → cholestenone + fatty acids + H_2O_2
H_2O_2 + catalase → H_2O + O_2 (no color development)
LDL-C + surfactant 2 + CE + CO + peroxidase + DSBmT → color development

2. Kyowa Medex selective solubilization method:
CM, VLDL, HDL + sugar compounds (α-cyclodextrin/dextran) + surfactants → complexes (blocks enzymes)
LDL-C + surfactant + CE + CO → cholestenone + H_2O_2
H_2O_2 + 4AAP + peroxidase + HDAOS → color development

3. Wako enzyme selective protection method:
LDL + "Compound Y" (modified PEG) → protects LDL from enzymes
CM, VLDL, and HDL + CE + CO → H_2O_2 + catalase (no color development)
LDL + "deprotecting reagent" + CE + CO → cholestenone + H_2O_2
H_2O_2 + 4AAP + peroxidase + HDAOS → color development

4. Denka Seiken elimination method:
Non-LDL + surfactant combination 1 + CE + CO → cholestenone + H_2O_2
H_2O_2 + catalase → H_2O (no color development)
LDL-C + surfactant combination 2 + CE + CO + sodium azide (to inhibit catalase) → cholestenone + H_2O_2
H_2O_2 + 4AAP + peroxidase + HDAOS → color development

5. Sysmex (formerly International Reagent Co) calixarene complex method:
LDL + calixarene → LDL-calixarene soluble complex
CM, VLDL, and HDL + CE1 + CD + hydrazine → cholestenone hydrazone
(CE from Chromobacterium viscosum cannot react with calixarene complexes)
LDL-C-calixarene complex + CE2 + CD + hydrazine + NAD + deoxycholate → cholestenone hydrazone + NADH

6. Serotec and UMA phosphate complex inhibition method:
LDL-C + detergent + phosphate compound + CE → free cholesterol
Free cholesterol + CO → cholestenone + H_2O_2
H_2O_2 + 4AAP + peroxidase + HDAOS → color development

Abbreviations: CM, chylomicrons; CE, cholesterol esterase; CO, cholesterol oxidase; CD, cholesterol dehydrogenase; 4AAP, 4-aminoantipyrine; DSBmT, N,N-bis-(4-sulfobutyl)-m-toluidine; HDAOS, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline; FDAOS, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoraniline.
Adapted from Nakamura M, Kayamori Y, Sato S, Shimamoto T. In: Focus on Cholesterol Research, MA Kramer, ed, Nova Science Publishers, Inc, New York, 2006.

The Kyowa assay measured 17% of VLDL cholesterol, 72% of IDL cholesterol, and 71-87% of Lp(a) cholesterol, as LDL cholesterol (19), while cholesterol associated with larger LDL was accurately measured, only 63% of small, dense LDL cholesterol was measured (19). Sensitivity and specificity issues were again evident in the Daiichi assay, which measured 24% of the VLDL cholesterol, 69% of the IDL cholesterol, and 80-91% of Lp(a) cholesterol, but only 45% of the small, dense LDL cholesterol (20).

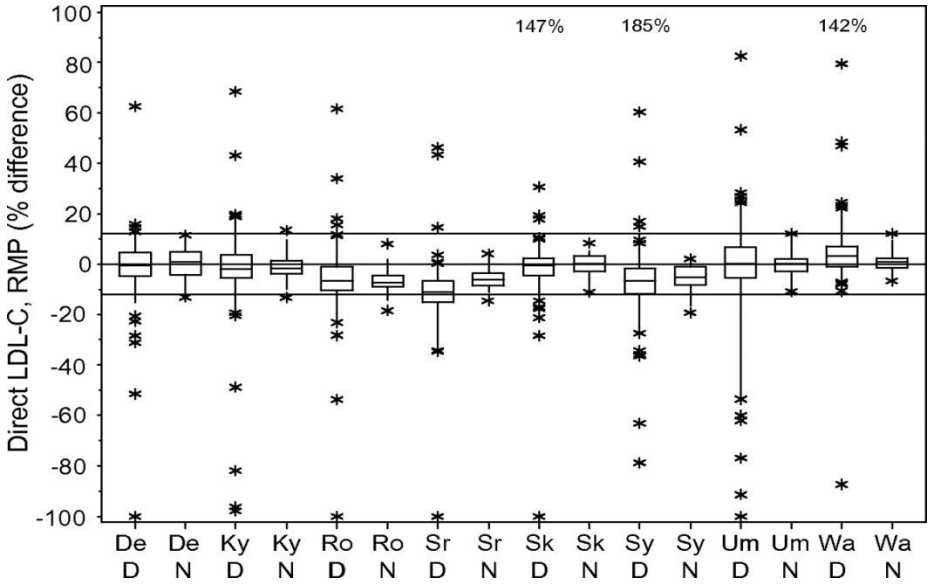
Bayer et al. determined that bias with many of the direct LDL-C methods was associated with VLDL cholesterol/triglyceride ratio (21), indicating that cholesterol enrichment of VLDL was a source of bias. This was also evident in a comparison of Roche and Wako assays that showed a 30% positive bias in individuals with Type III hyperlipoproteinemia (22).

In the most comprehensive study to date, Miller et al. compared direct methods for LDL cholesterol and HDL cholesterol with the BQ reference method using fresh samples from subjects with and without cardiovascular disease and/or various lipid and lipoprotein disorders (23). These data showed a dramatic lack of agreement with the reference methods, especially with specimens from patients with dyslipidemias and cardiovascular disease- so called "diseased" specimens. In fact, only five of the eight direct LDL cholesterol methods met the NCEP performance goals with specimens from nondiseased individuals and all methods failed to meet NCEP performance goals with those from diseased individuals (Figure 1 and Table 3) (23).

Errors in HDL Cholesterol

HDL cholesterol measurement is challenging because clinically important differences in concentrations are small and even small analytical error can contribute to misclassification. Like LDL cholesterol, the accuracy of HDL cholesterol is based on comparison with the CDC BQ method. An alternate designated comparison method (DCM) that does not require ultracentrifugation has also been developed to help standardize HDL cholesterol measurement through the Cholesterol Reference Method Laboratory Network (24). Separation techniques for HDL have included ultracentrifugation ($d>1.063$ kg/L), electrophoresis (α-migrating particles), and chromatography, but precipitation techniques were most popular before the introduction of homogeneous assays. Homogeneous (or direct) assays require no pretreatment or special handling. Unfortunately, as with direct LDL cholesterol assays, there appears to be poor agreement with reference methods and significant method-specific bias with these newer assays.

Figure 1: Box-and-Whisker Plot of the Differences in Percentage between the Direct and Reference Method Procedure Results for LDL-C for Each Direct Method



Abbreviations: D, diseased; N, nondiseased; De, Denka; Ky, Kyowa; Ro, Roche; Sr, Serotek; Sk, Sekisui; Sy, Sysmex; Um, UMA; Wa, Wako. From: Miller et al. Clin Chem 2010; 56:977-986.

Table 3: Total Error with Direct LDL Cholesterol Methods

LDL-C Assay	Non-Disease			Disease		
	Total CV, %	Mean Bias, %	Max TE*, %	Total CV, %	Mean Bias, %	Max TE*, %
Denka	6.2	0.2	13.5	10.5	-1.5	22.3
Kyowa	3.3	-1.1	-7.5	9.6	-0.8	20.4
Roche	3.8	-6.8	-13.3	10.0	-6.3	-23.3
Sekisui	4.2	-0.7	-8.8	6.0	-1.7	-13.5
Serotek	3.2	-6.2	-11.9	9.0	-11.8	-26.6
Sysmex	4.2	-6.0	-13.3	10.8	-7.8	-25.9
UMA	2.6	-0.1	5.3	13.8	-0.4	31.9
Wako	2.8	1.1	6.8	6.0	4.1	18.2

From Miller et al, Clin Chem 2010; 56:977-986; *Greater of positive or negative limit.

Precipitation Methods for HDL Cholesterol Measurement

All chemical precipitation methods are not equal. In addition to various reagents and concentrations, pH, ionic strength, and temperature may affect the separations. Typically, polyanions such as heparin, dextran sulfate, and sodium phosphotungstate are used with a divalent cation, such as magnesium, manganese, or calcium. Polyanions interact with arginine and other positively charged residues on apolipoproteins, while cations help the polyanion –lipoprotein complexes form via interaction with phospholipids. PEG, although not a polyanion, is also sometimes used to precipitate apo B-containing lipoproteins. Various concentrations of these components have been evaluated over the years to optimize the separation of HDL from other lipoproteins and to allow for the use of EDTA as well as serum. Additional magnesium or other divalent cation is necessary to account for the loss due to EDTA sequestration.

The dextran sulfate magnesium chloride precipitation method using dextran sulfate with an approximate molecular weight of 50,000, optimized to minimize HDL loss and maximize apo B lipoprotein precipitation, has been credentialed as an AACC Selected Method (25) and is the most popular precipitation method in the US.

Triglyceride-rich lipoproteins (TRL) in high concentrations may not completely precipitate, as evidenced by turbidity in the supernatant. Fortunately, this is easily observed and remedied. The sample can be filtered or centrifuged at high speed to remove the TRLs, or the specimen can be diluted with saline to dilute the TRLs. The precipitated sample may be salvaged by addition of an equal volume of saline and additional precipitation reagent. Centrifuging in a micro centrifuge at about 10,000 to 12,000 rpms for 5 minutes is better at clearing TRLs than traditional low speed centrifugation. Once precipitation is complete, total cholesterol measured in the supernatant is specific for HDL cholesterol. This is an important advantage, as assays for total cholesterol are better standardized and more accurate than homogeneous HDL cholesterol assays.

Second generation assays were developed to make HDL precipitation more efficient. These included a method that attached dextran sulfate to magnetic beads (LipiDirect) to allow easy separation of apo B-containing lipoproteins magnetically and without centrifugation (26). Another technique used a unique collection device (SpinPro) that collected the supernatant during centrifugation in a tube that could be transferred directly to an analyzer (26).

The Designated Comparison Method for HDL Cholesterol

The CDC reference method was the accuracy target for many influential population studies from which NCEP risk estimates and cut points were derived. However, the reference method is technically demanding and requires a 5 mL sample and an ultracentrifuge, which precludes its use as a routine reference method. The CDC and CRMLN laboratories have therefore developed and validated an alternative designated comparison method (DCM) based on precipitation with dextran sulfate and magnesium coupled with cholesterol measurement with the Abell-Kendall reference method. As shown below in Table 4, the DCM agrees very well with the reference method with a mean bias of 1.9% to 2.3% in participating laboratories (24).

Table 4: Performance of Candidate DCMs

Laboratory	Mean Bias	Mean Absolute Bias	Slope	Intercept (mg/dL)	r^2	Bias at 35 mg/dL	Bias at 60 mg/dL
Dextran Sulfate							
CDC	-0.4%	2.3%	1.08	-3.5	0.998	-2.3%	2.0%
PBRF	2.0%	2.3%	1.04	-0.9	0.997	1.5%	2.6%
WUSM	1.5%	1.9%	1.06	-1.9	0.998	0.5%	2.7%
Heparin-manganese							
CDC	0.2%	2.0%	1.06	-2.3	0.997	-1.0	1.7
PBRF	3.6%	3.8%	1.03	0.5	0.991	4.0	3.4
WUSM	1.4%	2.3%	1.08	-2.9	0.998	-0.2	3.3

Abbreviations: CDC: Centers for Disease Control and Prevention; PBRF: Pacific Biometrics Research Foundation; WUSM: Washington University School of Medicine (Seattle).
From: Kimberly et al. Clin Chem 1999; 45:1803-1812.

Direct HDL Cholesterol Methods

Third generation, homogeneous assays were a major breakthrough, allowing measurement of HDL cholesterol directly from the primary sample tube on a chemistry analyzer. Published reports generally showed good performance and potential for cost savings, mostly due to reduction in labor costs, although others report significant measurement errors (27-31). Like homogeneous LDL cholesterol assays, there are various methods for direct HDL cholesterol assays based on different

principles from different manufacturers (Table 5), and modifications to these methods over time make it difficult to assess current assay performance.

Lackner compared the Roche homogeneous assay to phosphotungstate magnesium precipitation in four patients with Type III hyperlipoproteinemia and found falsely elevated HDL cholesterol concentrations (32). Type III was diagnosed on the basis of total cholesterol and triglycerides >200 mg/dL, a ratio of VLDL cholesterol/total triglycerides >0.3, and apo E2 homozygosity. These data are summarized in Table 6. Roberts reported falsely low HDL cholesterol results in a patient with type III hyperlipoproteinemia (dysbetalipoproteinemia) with the Genzyme N-genous, the Roche HDL-C Plus, and the Sigma EZ HDL assays (33). Dextran sulfate and heparin precipitation provide correct results, while direct assays reported results that were 10% to 41% lower (Table 7). The reason for the discrepancy in results between these two studies is unclear, but may be related to the high background absorbance found with the specimen in Robert's case study (33).

Simo and colleagues compared the Daichii homogeneous assay and a PEG precipitation assay in five groups of patients; control subjects, healthy elderly, myocardial infarction patients, nephrotic syndrome patients, and liver cirrhosis patients. HDL cholesterol concentrations were significantly higher with the homogeneous assay compared with PEG precipitation in patients with nephrotic syndrome and cirrhosis (Table 8) (34). Camps et al. also compared HDL cholesterol measurement in 58 control subjects and 37 patients with cirrhosis, diagnosed by liver biopsy, using three homogeneous assays and ultracentrifugation (35). In this study the Daichii, Roche, and Sigma direct assays all gave falsely low results compared with HDL isolated by ultracentrifugation (Table 9). Interference from bilirubin and hemoglobin was also evident (35).

The most comprehensive study to date, reported by Miller et al (23), found that six of eight direct HDL cholesterol assays failed to meet NCEP total error goals in the healthy control group, while all eight assays failed to meet performance goals in the group with cardiovascular disease and/or lipoprotein disorders (Table 10, Figure 2) (23). Total variability ranged from 2.6% to 16.4% and total error ranged from -8.2% to 36.3%, and mean bias ranged from -8.6% to 8.8% between assays and the reference method (23).

Table 5: Direct HDL- Cholesterol Assays

1. Sekisui Medical (formerly Daiichi) accelerator selective detergent method:

CM, VLDL, LDL + synthetic polymers + polyanion → soluble complexes
HDL-C + selective detergent + CE + CO → cholestenone + fatty acids + H_2O_2
H_2O_2 + 4AAP + peroxidase + DSBmT → color development

2. Kyowa Medex modified enzymatic/selective detergent method:

CM, VLDL, LDL + α-cyclodextrin + magnesium chloride → soluble complexes
HDL-C + PEG-modified CE + CO → cholestenone + fatty acids + H_2O_2
H_2O_2 + 4AAP + peroxidase + DSBmT (or HDAOS?) → color development

3. Wako Pure Chemicals immunoinhibition method:

CM, VLDL, LDL + anti-apolipoprotein B → antigen-antibody soluble complexes
HDL-C + CE + CO → cholestenone + fatty acids + H_2O_2
H_2O_2 + 4AAP + peroxidase + FDAOS (or HDAOS?) → color development

4. Denka Seiken catalase elimination method:

CM-C, VLDL-C, LDL-C + CE + CO → cholestenone + fatty acids + H_2O_2
H_2O_2 + catalase → H_2O + O_2 (no color development)
HDL-C + detergent + CE + CO + sodium azide to inhibit catalase → cholestenone + fatty acids + H_2O_2
H_2O_2 + 4AAP + peroxidase (+ HDAOS) → color development

5. Sysmex (formerly International Reagent Co) calixarene complex method*:

CM, VLDL, LDL + calixarene → calixarene soluble complexes
HDL-C + CE + CD + hydrazine + NAD → cholestenone hydrazone + NADH
(CE from Chromobacterium viscosum cannot react with calixarene complexes)

6. Serotec and UMA phosphate complex inhibition method:

HDL-C + detergent + phosphate compound + CE → free cholesterol + fatty acids
Free cholesterol + CO → cholestenone + fatty acids + H_2O_2
H_2O_2 + 4AAP + peroxidase + HDAOS → color development

Abbreviations: CM, chylomicrons; CE, cholesterol esterase; CO, cholesterol oxidase; CD, cholesterol dehydrogenase; 4AAP, 4-aminoantipyrine; DSBmT, N,N-bis-(4-sulfobutyl)-m-toluidine; HDAOS, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline; FDAOS, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoraniline. *IRC also had an immunological method that may have been abandoned. Adapted from Nakamura M, Kayamori Y, Sato S, Shimamoto T. In: Focus on Cholesterol Research, MA Kramer, ed, Nova Science Publishers, Inc, New York, 2006 and Warnick et al. Clin Chem 2001; 47:1579-1596.

Summary

LDL and HDL are heterogeneous classes of particles that vary in size and composition, without a definitive chemical structure, making development of specific assays difficult. Direct assays based on different principles may measure different subsets of LDL or HDL, compounding the challenge of standardization. Direct LDL cholesterol and direct HDL cholesterol assays have not been adequately standardized, as evidenced by the bias between methods. Precipitation methods for HDL cholesterol measurement appear to provide more accurate results that direct methods and should be considered the method of choice for laboratories that desire accuracy.

As pointed out by Warnick, Nauck, and Rifai (26), "Laboratories supporting lipid clinics with a high proportion of specimens with atypical lipoproteins could observe discrepant results on certain specimens that might confound treatment decisions." It is important for these laboratories to choose assays wisely. I would also suggest that laboratories that support clinical trials, or conduct research related to lipid-related therapeutics or diagnostics, also choose methods that will best assess outcomes. For important measurements of HDL cholesterol, "old school" precipitation methods have no peer. For LDL cholesterol measurement homogeneous assays may not be the answer.

Table 6: Falsely Low HDL Cholesterol Concentrations in Four Patients with Dysbetalipoproteinemia

Method	Patient 1	Patient 2	Patient 3	Patient 4
Cholesterol, mg/dL	308	291	599	409
Triglycerides, mg/dL	440	503	595	529
VLDL-C/TG Ratio	0.37	0.36	0.66	0.52
HDL-Cholesterol, mg/dL				
Roche PEGME, serum	58	30	46	54
PTA/Mg, serum	30	14	ND	44
Roche PEGME, 1.006 infranate	ND	ND	34	44
PTA/Mg, 1.006 infranate	28	14	ND	ND

Adapted from: Lackner, Clin Chem 1998; 44:2546-2548.

Table 7: Falsely Low HDL-C in a Patient with Dysbetalipoproteinemia

Test	Result, mg/dL
Cholesterol	190
Triglycerides	282
HDL cholesterol, homogeneous	
N-geneous	7
HDL-C Plus	3
EZ HDL	12
HDL-cholesterol, precipitation	
Dextran sulfate/magnesium, serum	29
Dextran sulfate/magnesium, d>1.006 infranate	30
Heparin/manganese, d>1.006 infranate	30
HDL-cholesterol, REP Electrophoresis	31

Adapted from Roberts, Clin Chem 2000; 46:560-562.

Table 8: Comparison of the Daichii Homogeneous HDL Cholesterol Assay with PEG Precipitation in Five Patient Groups

	Controls	Healthy Elderly	AMI Patients	Nephrotic Syndrome Patients	Cirrhosis Patients
Total Cholesterol	5.47 ± 0.95	5.17 ± 1.09	5.56 ± 0.88	8.43 ± 3.49	5.04 ± 1.70
Triglycerides	1.70 ± 1.23	1.34 ± 0.47	1.96 ± 1.37	2.27 ± 1.50	1.22 ± 0.64
HDL-C, precipitation	1.10 ± 0.25	1.13 ± 0.32	1.07 ± 0.26	1.36 ± 0.23	1.25 ± 0.54
HDL-C, homogeneous	1.09 ± 0.27	1.12 ± 0.25	1.04 ± 0.30	1.26 ± 0.30	0.99 ± 0.36
Difference, %	-0.9	-0.9	-2.8	-7.4	-20.8

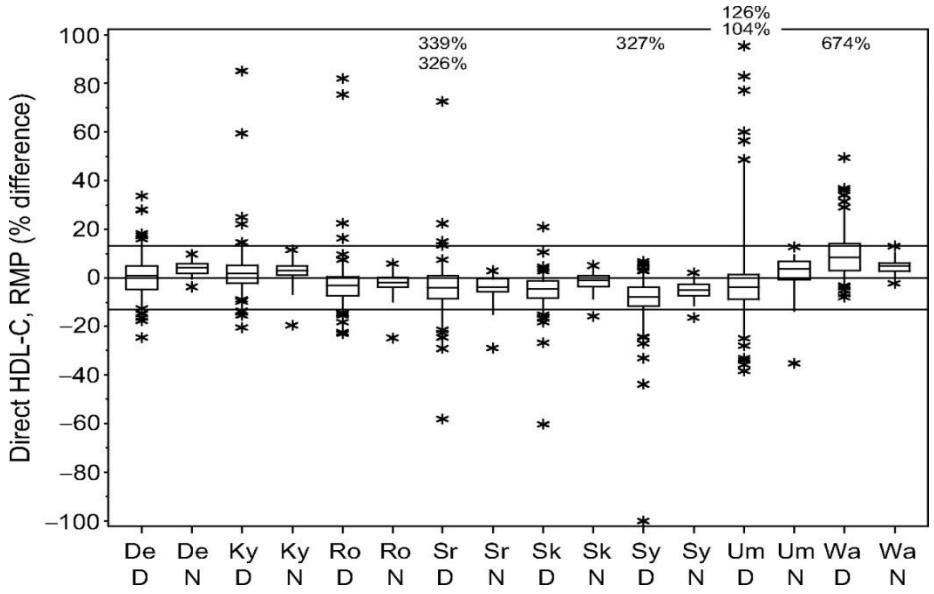
Adapted from Simo et al, Clin Chem 1998; 44:1233-1241.

Table 9: Falsely Low HDL-C in Cirrhotic Patients

Method	Cirrhotic Patients HDL-C, mg/dL	Controls HDL-C, mg/dL
Daichii (PPD)	39 (18-77)	46 (41-53)
Roche (PEGME)	29 (5-71)	47 (41-54)
Sigma (AB)	48 (27-90)	48 (41-55)
Ultracentrifugation	57 (19-134)	47 (41-53)

Adapted from Camps, Clin Chem 1999; 45; 685-688.

Figure 2: Box-and-Whisker Plot of the Differences in Percentage between the Direct and Reference Method Procedure Results for HDL-C for Each Direct Method



Abbreviations: D, diseased; N, nondiseased; De, Denka; Ky, Kyowa; Ro, Roche; Sr, Serotek; Sk, Sekisui; Sy, Sysmex; Um, UMA; Wa, Wako. From: Miller et al. Clin Chem 2010; 56:977-986.

Table 4: Total Error with Direct HDL Cholesterol Methods

HDL-C Assay	Non-Disease			Disease		
	Total CV, %	Mean Bias, %	Max TE, %	Total CV, %	Mean Bias, %	Max TE, %
Denka	2.9	4.0	10.4	8.4	0.4	18.8
Kyowa	3.7	2.5	10.4	8.1	2.1	20.0
Roche	4.3	-2.4	-10.4	8.1	-3.1	-17.5
Sekisui	3.4	-1.7	-8.2	6.1	-5.2	-16.0
Serotek	4.8	-4.8	-13.4	9.0	-3.0	-18.9
Sysmex	3.1	-5.4	-10.9	6.7	-8.6	-19.8
UMA	6.0	0.7	13.6	16.4	-1.9	36.3
Wako	2.6	4.8	10.5	6.4	8.8	24.0

From Miller et al, Clin Chem 2010; 56:977-986.
*Greater of positive or negative limit.

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