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Seven Direct Methods for Measuring HDL and LDL Cholesterol Compared with Ultracentrifugation Reference Measurement Procedures

W. Greg Miller,[1*] Gary L. Myers,[2] Ikunosuke Sakurabayashi,[3] Lorin M. Bachmann,[1] Samuel P. Caudill,[2] Andrzej Dziekonski,[1] Selvin Edwards,[2] Mary M. Kimberly,[2] William J. Korzun,[1] Elizabeth T. Leary,[4] Katsuyuki Nakajima,[5] Masakazu Nakamura,[6] Göran Nilsson,[7] Robert D. Shamburek,[8] George W. Vetrovec,[1] G. Russell Warnick,[9] and Alan T. Remaley[8]

BACKGROUND: Methods from 7 manufacturers and 1 distributor for directly measuring HDL cholesterol (C) and LDL-C were evaluated for imprecision, trueness, total error, and specificity in nonfrozen serum samples.

METHODS: We performed each direct method according to the manufacturer's instructions, using a Roche/Hitachi 917 analyzer, and compared the results with those obtained with reference measurement procedures for HDL-C and LDL-C. Imprecision was estimated for 35 runs performed with frozen pooled serum specimens and triplicate measurements on each individual sample. Sera from 37 individuals without disease and 138 with disease (primarily dyslipidemic and cardiovascular) were measured by each method. Trueness and total error were evaluated from the difference between the direct methods and reference measurement procedures. Specificity was evaluated from the dispersion in differences observed.

RESULTS: Imprecision data based on 4 frozen serum pools showed total CVs <3.7% for HDL-C and <4.4% for LDL-C. Bias for the nondiseased group ranged from −5.4% to 4.8% for HDL-C and from −6.8% to 1.1% for LDL-C, and for the diseased group from −8.6% to 8.8% for HDL-C and from −11.8% to 4.1% for LDL-C. Total error for the nondiseased group ranged from −13.4% to 13.6% for HDL-C and from −13.3% to 13.5% for LDL-C, and for the diseased group from −19.8% to 36.3% for HDL-C and from −26.6% to 31.9% for LDL-C.

CONCLUSIONS: Six of 8 HDL-C and 5 of 8 LDL-C direct methods met the National Cholesterol Education Program total error goals for nondiseased individuals. All the methods failed to meet these goals for diseased individuals, however, because of lack of specificity toward abnormal lipoproteins.

The current national guidelines on the use of LDL cholesterol (LDL-C)[10] and HDL-C for cardiovascular risk assessment *(1, 2)* were largely based on early epidemiologic studies that established the link between lipoproteins and cardiovascular disease. Most of these studies used chemical precipitation methods for HDL-C, and β-quantification or the Friedewald calculation for LDL-C. These older procedures have largely been supplanted by newer direct measurement methods for lipoprotein that do not depend on the physical separation of the different lipoprotein classes. The development of direct measurement methods was prompted, in part, by a recommendation from a National Cholesterol Education Program (NCEP) expert laboratory panel that stated LDL-C should ideally be directly measured *(3)* because of limitations of the Friedewald equation (4). A disadvantage of precipitation-based HDL-C methods was a requirement for manual pretreatment and centrifugation or some other type of labor-intensive separation step *(4)*. Homogeneous reagents for direct measurement of HDL-C and LDL-C facilitated automation and improved imprecision over the previous methods.

In 2008 there were 7 different commercial homogeneous direct measurement methods for HDL-C and LDL-C determination that were distributed worldwide under various trade names. These methods used a wide

[1] Virginia Commonwealth University, Richmond, VA; [2] US Centers for Disease Control and Prevention, Atlanta, GA; [3] Jichi Medical University, Tochigi-ken, Japan; [4] Pacific Biometrics and Pacific Biometrics Research Foundation, Seattle, WA; [5] Otsuka Pharmceutical, Tokyo, Japan; [6] Osaka Medical Center for Health Science and Promotion, Osaka, Japan; [7] Nilsson Measurement Quality, Uppsala, Sweden; [8] NIH, Bethesda, MD; [9] Health Diagnostics Laboratory, Richmond, VA.
* Address correspondence to this author at: PO Box 980286; Richmond, VA, 23298-0286. Fax 804-828-0353; e-mail gmiller@vcu.edu.
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[10] Nonstandard abbreviations: C, cholesterol; NCEP, National Cholesterol Education Program; RMP, reference measurement procedure; VCU, Virginia Commonwealth University; CDC, US Centers for Disease Control and Prevention.

variety of different surfactants, ionic polymers, and other components that either selectively prevented or enabled measurement of cholesterol in specific classes of lipoproteins among the full range of lipoprotein particles present in serum. Despite their advantages, there has been concern whether direct lipoprotein methods are analytically equivalent to older methods and to the established reference measurement procedures (RMPs) for HDL-C or LDL-C used as the basis for clinical guidelines.

For samples from dyslipidemic patients, who are higher-risk patients, measurement performance issues are of particular importance, especially when performance differences may affect clinical interpretation of results *(4–6)*. There have been many studies evaluating direct lipoprotein methods *(6–15)*, but these investigations usually examined only one or a few of these methods and in many cases did not compare the results to the RMPs. Samples from patients with dyslipidemias or other conditions known to challenge method performance were not always included in previous evaluations. In addition, the various direct lipoprotein methods have undergone considerable changes since their introduction, and older evaluation studies may not be relevant for the latest formulations of these methods.

This study was designed to evaluate direct measurement reagent formulations from 7 manufacturers for quantifying HDL-C and LDL-C. We examined patients with no known disease (nondiseased) as well as patients with known cardiovascular disease and other conditions previously shown to interfere with these methods (diseased), and we compared the results to those obtained with RMPs. We evaluated trueness, accuracy for individual samples, imprecision, and specificity for HDL and LDL lipoproteins, thus providing a comprehensive assessment of the analytical performance of the current direct lipoprotein methods.

Materials and Methods

PATIENT SAMPLES

Study participants were recruited at Virginia Commonwealth University Medical Center (VCU) and the NIH, with the approval of the respective institutional review boards. Of the 175 individuals enrolled (see Table S1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue6), 37 (21%) had no known diseases and were not on lipid-lowering drugs (nondiseased group) and 138 (79%) were being treated for cardiovascular disease or had other conditions that might be expected to affect lipoprotein methods (diseased group).

Blood was collected into plain glass vacuum tubes (Becton Dickinson), clotted 60–90 min, and centrifuged (1800*g* for 10 min at 4 °C). Serum from each study participant was combined and mixed in plastic vials before being divided into aliquots. Two full 6-mL aliquots in plastic tubes were shipped overnight at 4 °C in insulated shipping containers, containing temperature indicators (#310 with #319 refrigerant, Saf-T-Pak), to either the US Centers for Disease Control and Prevention (CDC) or to VCU. Samples were measured by direct methods (VCU) and RMPs (CDC) within 48 h after collection, and all measurements were performed on the same day. Because of unacceptable QC parameters RMP values were not available for 1 HDL-C and 3 LDL-C individual sample measurements.

DIRECT HDL-C AND LDL-C METHODS

Reagents, calibrators, and verification controls were provided by Denka Seiken, Niigata; Kyowa Medex, Tokyo; Sekisui Medical, Tokyo (formerly Daiichi); Serotec, Hokkaido; Sysmex International Reagents, Hyogo; UMA, Shizuoka; and Wako Pure Chemical Industries, Osaka (all located in Japan) and were distributed worldwide under various trade names by instrument manufacturers and reagent suppliers. Roche Diagnostics, Penzberg, Germany, the distributor of Kyowa Medex reagents, also provided reagents with Roche calibrators and controls.

All methods were performed on a Hitachi 917 analyzer (Roche Diagnostics), according to parameters recommended by each manufacturer. Each method was installed on a separate channel and calibrated weekly, and method-specific controls were verified to meet each manufacturer's specifications, except for UMA, which did not provide verification controls. Reagents and calibrators from a single lot were used for the Denka Seiken, Kyowa Medex, Sekisui Medical, and Roche methods; reagents from 2 lots were used for the Serotec, Wako, and UMA methods; and reagent from 1 lot and calibrators from 2 lots were used for the Sysmex method. We verified each lot to conform to the manufacturer's analytical range by recovering a linear concentration relationship for patient sera prepared by mixing samples with increased and low concentrations of each analyte. In addition to the normal wash (Cell Wash Solution, Roche Diagnostics), the reagent probe and each reaction cell underwent an additional wash with 300 μL of Acid Wash Solution (Roche Diagnostics) between each measurement to minimize reagent carryover. We verified that instrument pipette and spectrophotometer CVs were <0.5% weekly, by using a solution of 0.068 mol/L potassium dichromate in 0.005 mol/L H_2SO_4, with 0.145 mol/L NaCl and 1% (vol/vol) Hitergent® (Roche Diagnostics).

Aliquots of 4 off-the-clot frozen human serum pools, prepared according to the CLSI C37A protocol *(16)*, were provided by the CDC and measured in duplicate before and after each run. Frozen pools and patient samples were measured in the sequence shown in online Supplemental Table S2. A maximum of 8 patient samples were measured in a run, which required 2 h to complete all methods.

REFERENCE MEASUREMENT PROCEDURES

The reference measurement procedures for HDL-C and LDL-C were performed at the CDC *(17)*. Chylomicrons and VLDL were removed by ultracentrifugation of 5 mL of serum, overlaid with 0.195 mol/L NaCl, for 16.2 h at a mean of 120 000*g* (33 700 rpm) in a Beckman-type 50.4 rotor at 18 °C. The top layer, which contained chylomicrons and VLDL components, was removed by slicing the tube. The remaining bottom fraction containing HDL and LDL was quantitatively transferred to a 5-mL volumetric flask, and the volume was made up to 5 mL with 0.15 mol/L sodium chloride. The cholesterol in this bottom fraction was analyzed by the Abell-Kendall RMP *(18)*. For HDL-C measurements, a 2-mL aliquot of the bottom fraction was precipitated with 80 μL of injectable heparin (5000 USP units/mL plus 0.15 mol/L NaCl in water) and 100 μL of 1.0 mol/L manganese chloride in water to remove lipoproteins that contained apolipoprotein B. We measured the HDL-C in the supernatant by using the RMP for cholesterol. Beta-quantification LDL-C was obtained by subtracting the HDL-C concentration from the corresponding bottom-fraction cholesterol concentration. Ultracentrifugation was performed in duplicate, except for 21 samples for which insufficient serum was available. Duplicate cholesterol reference measurements were performed for each bottom fraction and HDL supernatant (net 4 HDL-C or LDL-C results for each sample, except 18 of 696 HDL supernatant measurements and 36 of 688 bottom-fraction measurements that had 3 results each owing to outlier exclusions).

OTHER METHODS

Measurement procedures for other analytes and statistical procedures, including estimation of error components, definition of error component terms, and evaluation of the Genzyme (Cambridge, MA) calibrator for HDL-C reagents, are described in the online Supplemental Data file.

Results

IMPRECISION

The imprecision of each method was determined from 4 frozen serum pools (see online Supplemental Table S4). The total CV for HDL-C on the individual frozen serum pools ranged from 1.5% (Sekisui) to 3.7% (Sysmex) and was below the HDL-C imprecision goal of ≤4%. All the LDL-C methods showed imprecisions with total CVs ranging from 1.4% (Kyowa) to 3.7% (Serotec), with only 1 pool (#715) with 1 method (Serotec) having a CV of 4.4%, which exceeded the LDL-C imprecision goal of ≤4%.

Online Supplemental Figs. S1–S16 show plots for the 4 frozen serum pools on each day of measurement over the 28-week study. For LDL-C, there was a shift in results for Serotec (3.6% to 7.5%) and UMA (−4.9% to −2.1%) methods concurrent with reagent-lot changes, and a shift in the Sysmex results (−3.9% to 3.3%) concurrent with a calibrator-lot change. The influences of among day effects and reagent- and calibrator-lot changes are included in the imprecision statistics based on frozen pools.

Online Supplemental Tables S5 and S6 show differences in mean results for the frozen pools measured at the beginning and end of each 2-h run. The magnitude of change from beginning to end of HDL-C runs ranged from −1.0% to 3.4% and for LDL-C ranged from −0.4% to 3.5%, with more frequent observation of higher values at the end of runs. The position effects are included in the imprecision statistics based on frozen pools.

TRUENESS AND TOTAL ERROR

Figs. 1 and 2 show box-and-whisker plots for the nondiseased and diseased groups for the percentage differences between the means of triplicate measurements of each sample and its RMP value for the HDL-C and LDL-C methods, respectively. The median difference in the nondiseased group is a close approximation of the trueness of the calibration, whereas the median difference is influenced by nonspecificity in the diseased group. The interquartile distance and range are related to the lipoprotein specificity and total error. As can be seen for all the methods, there were considerably more discordant results for the diseased vs nondiseased study participants. Sysmex and Wako HDL-C methods showed consistent negative and positive biases, respectively, compared to the RMP. For LDL-C, the Roche, Serotec, and Sysmex methods showed a consistent negative bias.

Online Supplemental Figs. S17–S32 show plots of the percentage differences vs the RMPs for each method. Nearly all discordant results occurred in the diseased group. Except for the UMA methods, which had discordant results throughout the concentration range for both HDL-C and LDL-C, most of the other methods had discordant results mostly at lower HDL-C (<1 mmol/L; <40 mg/dL) and LDL-C (<2.5 mmol/L; <100 mg/dL) concentration ranges.

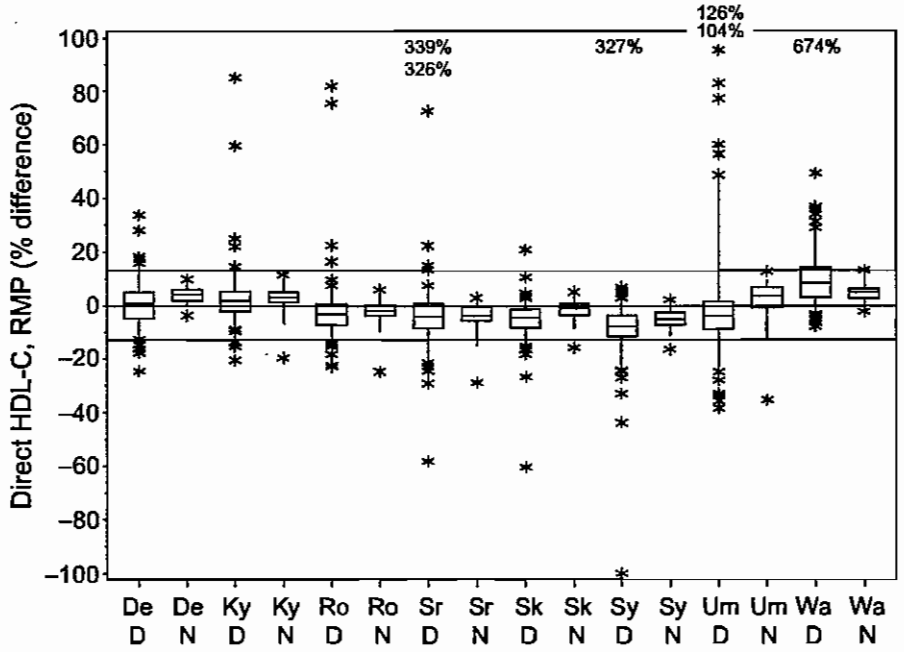

Fig. 1. Box-and-whisker plot of the differences in percentage between the direct and RMP results for HDL-C for each direct method (D, diseased group; N, nondiseased group; De, Denka; Ky, Kyowa; Ro, Roche; Sr, Serotek; Sk, Sekisui; Sy, Sysmex; Um, UMA; and Wa, Wako).

The median is the center line, the ends of the box represent 25th and 75th percentiles, the end of the lines extend to the 10th and 90th percentiles, and individual results are shown beyond the lines.

TOTAL ERROR FOR SINGLE MEASUREMENTS

The total error for a single measurement was based on the first result (no replication) for each sample. The percentage of results (without exclusions) for each method that were within the NCEP total error goals are shown in Table 1. For nondiseased individuals, most of the HDL-C methods reached the 95% acceptance criterion *(3)*, and only the Serotec and UMA methods did not meet this criterion. The performance of all the HDL-C methods considerably deteriorated for the samples from the diseased group. Only the Roche and Sekisui methods for HDL-C met the total error goals for the diseased study patients. In general, the performance of the LDL-C methods was not as good as the HDL-C methods for both the diseased and nondiseased samples, with 4 of the LDL-C methods not meeting the criterion for the nondiseased individuals and all the methods failing for the diseased individuals. For the Serotec LDL-C method, only slightly more than half the results for the diseased study participants were within the NCEP total error goal.

ERROR SOURCES CONTRIBUTING TO TOTAL ERROR

Table 2 shows the contribution of different major sources of error for the nondiseased and diseased groups. Note that some markedly discordant results, frequently from the same individuals, were excluded as outliers for the error component analysis to avoid any disproportionate influence on mean bias or CV attributable to patient sample specific effects (CV_d; see online Supplemental Data for details). Thus, the error components listed in Table 2 may be underestimated in some cases.

For the nondiseased group, 6 of 8 HDL-C methods met the NCEP total error goal of ≤13%. The Serotec method and the UMA method, however, minimally exceeded this limit, primarily owing to increased imprecision from sample-specific effects (CV_d). In contrast, all of the HDL-C methods exceeded the total error goal for the diseased individuals, sometimes by substantial amounts. Most of the excess error was attributable to sample-specific effects (CV_d), most likely due to inadequate specificity for the HDL-C lipoprotein class, but several of the methods also exceeded the recommended mean bias of ≤5%.

For the LDL-C methods, 5 of 8 met the NCEP total error goal of ≤12% for the nondiseased group. The Denka, Roche, and Sysmex methods minimally exceeded the total error goal owing to a combination of imprecision and bias. All LDL-C methods substantially

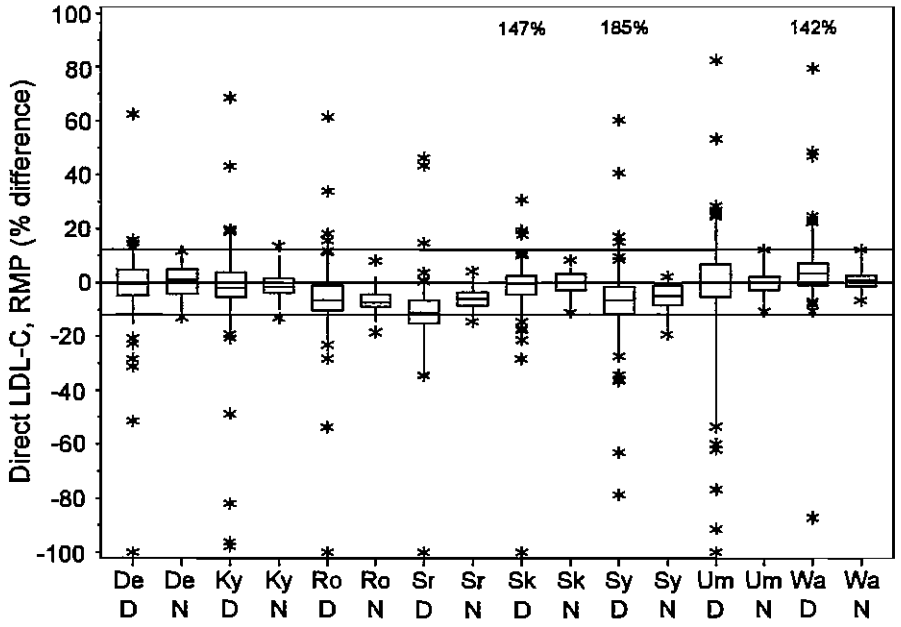

Fig. 2. Box-and-whisker plot of the differences in percentage between the direct and RMP results for LDL-C for each direct method (abbreviations as defined in the Fig. 1 legend).

The median is the center line, the ends of the box represent 25th and 75th percentiles, the end of the lines extend to the 10th and 90th percentiles, and individual results are shown beyond the lines.

exceeded the total error goal for the diseased individuals, primarily because of sample-specific effects (CV_a). Three of the LDL-C methods showed a negative mean bias exceeding the goal of ≤4% for both the nondiseased and diseased groups.

SPECIFICITY ISSUES FOR CLASSES OF INTERFERING SUBSTANCES

To investigate an association between triglyceride concentrations and discordant results, all results were combined and grouped into triglyceride tertiles (Figs. 3 and 4). Five of 8 HDL-C methods were found to have a

Table 1. Percentage of results within the NCEP total error (TE) requirements for a single measurement of each patient's serum.[a]

Method	%HDL-C results within TE requirement,[b] nondiseased	%HDL-C results within TE requirement, diseased	%LDL-C results within TE requirement,[c] nondiseased	%LDL-C results within TE requirement, diseased
Denka	100	92.7	89.2	85.2
Kyowa	97.3	94.2	94.6	85.9
Roche (Kyowa)	97.3	95.6	91.9	77.0
Sekisui	100	96.4	100	91.1
Serotec	94.6	86.9	97.3	53.3
Sysmex	100	89.8	86.5	71.9
UMA	91.9	83.9	97.3	75.6
Wako	100	74.5	97.3	87.4

[a] Determined from the first result (not the mean of triplicate measurements) for each study participant. No study participants were excluded.

[b] Total error criteria for HDL-C are: 95% of results ≤13% (with desirable imprecision ≤4% and bias ≤5%) when HDL-C is ≥1.09 mmol/L (≥42 mg/dL), and, when HDL-C is <1.09 mmol/L (<42 mg/dL), within a percentage determined by using a fixed 0.044 mmol/L (1.7 mg/dL) SD in the equation: 5% bias + [(1.96 × 0.044 mmol/L × 100)/concentration (in mmol/L)].

[c] Total error criteria for LDL-C are: 95% of results ≤12% (with desirable imprecision ≤4% and bias ≤4%).

Table 2. Error components[a] for direct HDL-C and LDL-C methods.

Method	CV_b, %, interassay, frozen pools	CV_e, %, intraassay, patient samples	CV_d, %, patient sample–specific effects	CV_{tot}, %, combined random effects of CV_b, CV_e, and CV_d	Mean bias, %	TE, %, for greater of positive or negative limit
HDL-C, nondiseased group						
Denka	1.4	1.2	2.3	2.9	4.0	10.4
Kyowa	0.9	0.9	3.5	3.7	2.5	10.4
Roche	1.4	1.4	3.8	4.3	−2.4	−10.4
Sekisui	0.9	1.1	3.1	3.4	−1.7	−8.2
Serotec	1.9	1.2	4.2	4.8	−4.8	−13.4
Sysmex	2.3	1.0	1.8	3.1	−5.4	−10.9
UMA	1.1	1.2	5.8	6.0	0.7	13.6
Wako	1.7	1.3	1.4	2.6	4.8	10.5
HDL-C, diseased group[b]						
Denka		1.2	8.2	8.4	0.4	18.8
Kyowa		1.8	7.8	8.1	2.1	20.0
Roche		2.0	7.7	8.1	−3.1	−17.5
Sekisui		1.2	5.9	6.1	−5.2	−16.0
Serotec		1.7	8.6	9.0	−3.0	−18.9
Sysmex		1.6	6.1	6.7	−8.6	−19.8
UMA		1.9	16.3	16.4	−1.9	36.3
Wako		1.2	6.0	6.4	8.8	24.0
LDL-C, nondiseased group						
Denka	1.3	2.8	5.4	6.2	0.2	13.5
Kyowa	0.7	0.7	3.2	3.3	−1.1	−7.5
Roche	0.8	1.6	3.3	3.8	−6.8	−13.3
Sekisui	1.2	1.5	3.8	4.2	−0.7	−8.8
Serotec	2.9	1.4	0.0	3.2	−6.2	−11.9
Sysmex	2.3	0.7	3.4	4.2	−6.0	−13.3
UMA	2.2	0.9	1.2	2.6	−0.1	5.3
Wako	0.6	1.8	2.0	2.8	1.1	6.8
LDL-C, diseased group[c]						
Denka		2.2	10.5	10.8	−1.5	22.3
Kyowa		1.1	9.6	9.7	−0.8	20.4
Roche		1.3	10.0	10.1	−6.3	−23.3
Sekisui		2.0	6.0	6.4	−1.7	−13.5
Serotec		1.3	9.0	9.5	−11.8	−26.6
Sysmex		0.9	10.8	11.1	−7.8	−25.9
UMA		1.5	13.8	14.1	−0.4	31.9
Wako		1.8	6.0	6.3	4.1	18.2

[a] See online Supplemental Data for detailed explanation of error component terms, criteria for outlier exclusion, and calculation of total error from the estimated component contributions.

[b] HDL-C, diseased group results excluded and difference value: sample 108, 0.99 mmol/L (38 mg/dL), from UMA (126%); sample 220, 0.66 mmol/L (25 mg/dL), from Serotec (−58%); sample 310, 0.1 mmol/L (4 mg/dL), from Sekisui (−60%), Serotec (339%), Sysmex (327%), Wako (674%); sample 348, 0.33 mmol/L (13 mg/dL), from Serotec (326%), Sysmex (−100%, <LOQ).

[c] LDL-C, diseased group results excluded and difference value: sample 101, 2.95 mmol/L (114 mg/dL), from UMA (−60%); sample 102, 4.20 mmol/L (162 mg/dL), from UMA (−62%); sample 108, 4.44 mmol/L (172 mg/dL), from UMA (−77%); sample 145, 0.78 mmol/L (30 mg/dL), from Serotec (−100%, <LOQ); sample 178, 0.81 mmol/L (31 mg/dL), from Serotec (−100%, <LOQ); sample 220, 0.67 mmol/L (26 mg/dL), from Sysmex (−63%); sample 310, 2.56 mmol/L (99 mg/dL), from Kyowa (−98%), Roche (−100%, <LOQ), Serotec (−100%, <LOQ), Sysmex (−79%), UMA (−91%), Wako (−87%); sample 348, 0.60 mmol/L (23 mg/dL) and very lipemic, Kyowa (−96%), Roche (−100%, <LOQ), Sekisui (147%), Serotec (−100%, <LOQ), Sysmex (185%), UMA (−100%, <LOQ), Wako (142%); sample 349, 0.07 mmol/L (2.6 mg/dL), all methods.

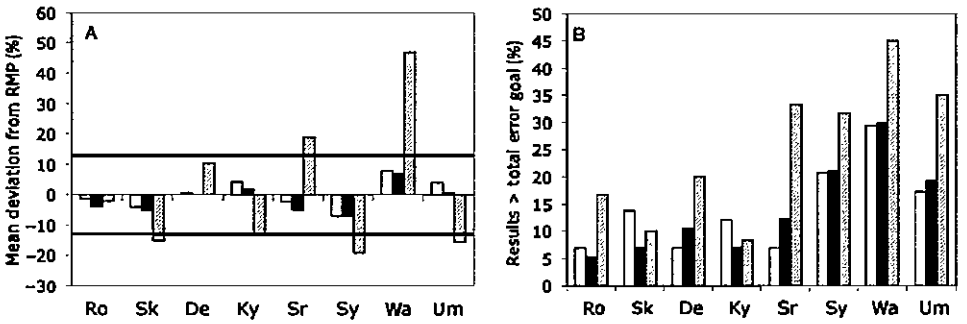

Fig. 3. Percentage mean deviation from RMP values (A) and the percentage of direct method HDL-C results greater than the NCEP total error goal (B) for samples grouped by tertiles of triglycerides (TG) concentrations (abbreviations as defined in the Fig. 1 legend).

Open bars TG <0.94 mmol/L (83 mg/dL); shaded bars TG 0.94 mmol/L (83 mg/dL) to 1.60 mmol/L (142 mg/dL); striped bars TG >1.60 mmol/L (142 mg/dL). The solid lines in (A) represent the total error goal as ±13% from the RMP. Note the total error goal becomes larger at HDL-C concentrations below 1.09 mmol/L (42 mg/dL) (see footnote in Table 1), and this criterion was used in the calculation for (B).

mean bias that exceeded the total error goal for the high triglyceride group. Increased triglycerides caused either a positive or negative bias, depending on the method. Furthermore, the frequency of discordant cases that exceeded the NCEP total error goal was usually smallest in the low or middle tertile and greatest in the high tertile. For LDL-C, higher triglycerides were associated with an increase in the direct LDL-C results compared to the RMP, except for the Serotec method, which had a mean negative bias exceeding the total error goal for the high tertile. For the low triglycerides tertile, 7 of 8 LDL-C methods showed a negative bias, with the Roche method exceeding the total error goal. No consistent pattern was observed for the frequency of discordant LDL-C results with triglyceride concentration.

We also inspected the clinical information for all study participants whose samples had a result that differed from the RMPs by ≥20% (see online Supplemental Tables S7 and S8). For HDL-C, all but 1 of 42 study participants came from the diseased group. All of these individuals except 3 had cardiovascular disease and/or a known dyslipidemia. All 40 study participants with discordant LDL-C results came from the diseased group and all except 2 had cardiovascular disease and/or dyslipidemia.

There were only a limited number of study participants with increased IgG (range 20.0–44.2 g/L, n = 4),

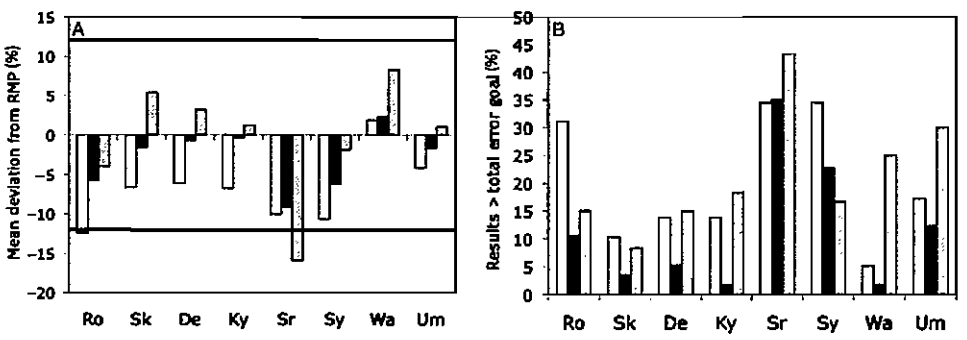

Fig. 4. Percentage mean deviation from RMP values (A) and the percentage of direct method LDL-C results greater than NCEP total error goal (B) for samples grouped by tertiles of triglyceride concentrations (abbreviations as defined in the Fig. 1 legend).

The solid lines in (A) represent the total error goal as ±12% from the RMP.

total bilirubin (range 26–58 μmol/L [1.5–3.4 mg/dL], n = 7), or alanine aminotransferase (range 83–110 U/L, n = 4), making it difficult to fully assess interferences from conditions associated with these analytes. One patient with end-stage renal disease who was receiving dialysis treatment, with 37.7 g/L IgG and 25 g/L albumin, had HDL-C values that were 16% to 58% lower than the RMP and LDL-C values that were 22% to 63% lower than the RMP for the different methods, except for the Roche HDL-C and LDL-C methods, which showed no discrepancy with RMP values. Other study participants in this subpopulation had HDL-C and LDL-C results <20% different from the RMPs.

Discussion

The NCEP accuracy goals for HDL-C and LDL-C were based, in part, on the state of the art of laboratory testing at the time the guidelines were developed, and on clinical need for accurate classification of chronic heart disease risk and monitoring of lipid treatment goals. Numerous studies have shown that these accuracy goals were largely met with the precipitation-based HDL-C methods *(19)*, but because errors were compounded in the multiple analytes used for calculating LDL-C by the Friedewald equation, as well as other limitations, these methods were considered unsatisfactory *(3, 4)*.

The composition of lipoproteins in various dyslipidemias influences the ability of direct methods to specifically measure the cholesterol content of one lipoprotein class in the presence of other types of lipoproteins. Consequently, it has been challenging to manufacture direct methods with adequate specificity, because a large number of factors related to genetics, nutrition, disease, and treatment affect the composition of lipoproteins. The direct methods performed well for the nondiseased group, but they all had unacceptable total error for the diseased group (Tables 1 and 2). The primary contributing error components were sample-specific influences. The most likely limitation was nonspecificity for the intended lipoprotein density fraction in the presence of abnormal lipoproteins. Measurements for samples with high or low triglycerides, in particular, were challenged to agree with the RMPs (Figs. 3 and 4). The concentrations of triglycerides in the various lipoprotein fractions are known to be highly variable and to change with lipid disorders and other conditions *(20)*. It is not possible, however, to rule out the potential influence of other interfering substances that may have been present in some of the diseased study participants, most of whom were taking a number of drugs and had various comorbidities. Nonfasting samples, which, according to the manufacturers, are acceptable for use in the direct methods, likely introduced additional confounders that were not investigated in this study.

In many cases the differences between the direct method results and RMPs were sufficiently large (Figs. 1 and 2; Table 2) that they could affect the clinical management of patients. Many of the discordant cases were individuals with low HDL-C, which frequently occurs with hypertriglyceridemia *(21)*. Several patients with a genetic disorder in lipid metabolism, such as LCAT deficiency, had direct-method results that differed so markedly from the RMPs and from the typical values seen in these kinds of patients with HDL-C precipitation–based methods and a calculated LDL-C that they could have been misdiagnosed. Inaccurate HDL-C results may also lead to incorrect cardiovascular risk assessments and to improper choices in drug therapy.

Many of the discordant results were present at lower analyte concentrations. Given the recent interest in more aggressively treating individuals with drugs to lower LDL-C below 1.8 mmol/L (70 mg/dL) to reverse existing atherosclerotic disease *(22)*, accurate measurement of low LDL-C will likely become more important in the future. It is important to note that for some direct methods 30%–45% of test results fell outside the NCEP total error goals for the diseased group (Table 1), which would be expected to reduce the overall effectiveness of screening for cardiovascular risk assessment by direct HDL-C or LDL-C measurements. It is important to note that the frequency and magnitude of the errors observed in this study may be different in other populations that may have different types of dyslipidemias. Several of the patients recruited from the NIH had rare genetic lipid disorders, and the results from these patients may not be representative of the typical performance of the direct methods. However, the majority of the other patients from the NIH and from VCU, who were recruited from a cardiology clinic, had more common forms of lipid disorders (see online Supplementary Tables S1, S7, and S8).

Strengths of this investigation include examination of a range of individuals without disease and patients with various types of lipoprotein disorders and other diseases known to have caused errors in earlier generations of direct measurement reagents. Direct measurement reagents from 7 primary manufacturers were included, and measurements with each method were made at the same time with a single automated analyzer. The RMPs were unmodified and performed by the CDC. Blood collection and processing to obtain serum was performed in the same manner as is typical for clinical testing, and all measurements, including the RMPs, were performed within 2 days of collection on sera stored at 4–8 °C. Sufficient data were available to use an error component model to determine the relative contributions to total error from various sources.

The error component model avoided biased estimates by using differences of ln(concentration) instead of relative percentage differences.

Limitations of this investigation include small changes in results for frozen serum controls from beginning to end of some runs for the direct methods (see online Supplemental Tables S5 and S6 and Supplemental Figs. S1–S16). Examination of the measurement sequence (see online Supplemental Table S2) indicated that any evaporation would not have affected results for patient samples, but may have contributed to the observed CVs for the frozen serum pools. However, the interassay (from controls) and intraassay (from patient sample replicates) CVs (Table 2) were similar for each method, suggesting the magnitude of changes during a run was not an important influence on conclusions. Reagent and calibrator lot changes were not systematically evaluated; however, there were apparent influences of reagent lot on frozen serum pool results for 2 of 3 LDL-C methods that had reagent-lot changes during the study.

A limitation in the error component analysis included exclusion of some outliers either because the values were 0 (logarithm of 0 not possible) or the values were highly discrepant from the RMPs (difference of ln(concentration) exceeded ±0.8). Outlier exclusion criteria were somewhat arbitrary; those chosen gave reasonable estimates of dominating error sources while excluding results that would have excessively influenced bias and SD terms.

The current NCEP Adult Treatment Panel recommendations for cardiovascular risk assessment were based on epidemiologic results, with use of the Friedewald equation to estimate LDL-C, and HDL-C methods that produced results in agreement with the RMP. Direct HDL-C and LDL-C methods have been used with the same decision points; however, the relationship of direct method results to cardiovascular risk assessment has not been systematically investigated.

In summary, 6 of 8 HDL-C and 5 of 8 LDL-C direct methods met the NCEP total error goals for nondiseased individuals, but all direct methods failed to meet these goals for samples from patients with cardiovascular disease and/or dyslipidemia. Sample-specific effects were the dominant cause of discrepant results.

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