Improvement in Japanese Clinical Laboratory Measurements of Total Cholesterol and HDL-cholesterol by the US Cholesterol Reference Method Laboratory Network

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Background: Accurate and precise measurements of total cholesterol (TC) and HDL-cholesterol (HDL-C) are necessary for effective diagnosis and treatment of lipid disorders. We studied the impact of TC certification and HDL-C evaluation in Japanese clinical laboratories to standardize their measurements.

Methods: We selected 78 laboratories participated at least twice for TC and 46 laboratories participated twice for HDL-C in the standardization protocols developed by the Cholesterol Reference Method Laboratory Network (CRMLN). We compared the initial and subsequent results using the performance guidelines established by US National Cholesterol Education Program (NCEP).

Results: For TC, mean percentage bias of all participants was -0.93% and -0.49% for the initial and second rounds, respectively. Mean within-sample CV was 0.72% and 0.69% for the initial and second rounds, respectively. For HDL-C, mean percentage bias of all participants was -1.86% and -0.06% for the initial and second events, respectively. Mean among-run CV was 1.56% and 1.58% for the initial and second events, respectively.

Conclusions: TC accuracy in the second round than the initial round tended to improvement although statistically not significant, however in the five years follow-up, mean absolute percentage bias was reduced over time. HDL-C accuracy was statistically improved in the second event than the initial event. The precision for both TC and HDL-C did not change. This study shows CRMLN protocols contribute effectively to improvement of TC and HDL-C performance. *J Atheroscler Thromb*, 2003; 10: 145-153.

Key words: Total cholesterol, HDL-cholesterol, Accuracy, Precision

Introduction

Research for epidemiological studies and clinical trials have demonstrated that high TC and/or high LDL-cholesterol (LDL-C) are an important risk factor for coronary heart disease (CHD) (1-5) and that low HDL-C is an in-

dependent predictor of risk for CHD (6-8). According to the recent studies LDL-lowering therapy robustly reduces risk for CHD (9-13).

Guidelines for diagnosis and treatment of lipid disorders were issued in three reports from the US NCEP Adult Treatment Panel (ATP) in 1988 for ATP-I, in 1993 for ATP-II and in 2001 for ATP-III (14). The European Atherosclerosis Society (EAS) issued similar guideline in 1998 (15). The Japan Atherosclerosis Society (JAS) issued the first guideline in 1997 and updated it in 2002 (16). To identify individuals at risk for CHD, the NCEP recommends initial classification in ATP-III using the medical decision points

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of 5.17 and 6.21 mmol/L (200 and 240 mg/dL) for TC and of 1.03 mmol/L (40 mg/dL) for HDL-C, and the JAS recommends initial classification in 2002 revision using the medical cut points of 5.69 mmol/L (220 mg/dL) for TC and of 1.03 mmol/L (40 mg/dL) for HDL-C. Any recent guideline emphasizes the importance for measurement of LDL-C rather than TC.

Accurate, reproducible, and comparable measurements of TC and HDL-C are needed for effective application of the guidelines. The NCEP recommendations indicate that the performance goals for TC are accuracy, expressed as percentage bias versus the accuracy base, of $\pm 3\%$ and imprecision, expressed as CV, of $\leq 3\%$ (17). The performance goals for HDL-C are accuracy, expressed as percentage bias versus the accuracy base, of $\leq \pm 5\%$ and imprecision, expressed as CV, of $\leq 4\%$ at HDL-C concentrations > 1.09 mmol/L (42 mg/dL) and as standard deviation ≤ 0.044 mmol/L (1.7 mg/dL) at HDL-C ≤ 1.09 mmol/L (42 mg/dL) (18, 19).

In 1990, the CDC established the CRMLN (20,21) to improve lipid and lipoprotein measurements by providing traceability to the accuracy bases for these analytes. In addition to US-based laboratories, CDC, in its role as a World Health Organization Collaborating Center for Reference and Research in Blood Lipids, extended the CRMLN to include selected international laboratories. The Osaka Medical Center for Health Science and Promotion (OMC) has been a member of the CRMLN since July 1992. The CRMLN developed a process by which manufacturers and clinical laboratories can establish traceability to the US National Reference System for Cholesterol (NRS/CHOL), as recommended by NCEP. In 1995, this process was extended to manufacturers producing products used to measure HDL-C (22).

Traceability for TC in a clinical laboratory is verified by comparing the field method with the Abell, Levy, Brodie and Kendall (AK) reference method for TC (23, 24) in a CRMLN laboratory. The comparison is performed based on the CRMLN's Certification Protocol for Clinical Laboratories (25). For HDL-C, the CRMLN has not yet established a specific protocol for certifying clinical laboratories. We therefore applied the CRMLN protocol for certifying manufacturers to Japanese clinical laboratories. The protocol for manufacturers involves a comparison between the field method and the designated comparison method (DCM) for HDL-C using a minimum of 40 to 50 fresh human specimens (26).

The impact of standardization in clinical laboratories has not been well documented. We report here the results for the effectiveness of the CRMLN's TC and HDL-C protocols toward improvement of Japanese clinical laboratory measurements. They participated in the certification process on a voluntary basis. At the same time, to know the effectiveness of long-term standardization, we

also report the results based on 5 years of follow-up comparisons for TC.

Materials and Methods

Reference methods

The AK reference method (23, 24) consists of saponification of a 0.5-mL serum sample with alcoholic potassium hydroxide, extraction with hexane, evaporation of an aliquot of the extract, development of color with Liebermann-Burchard reagent at 620 nm, and calibration by the NIST SRM 911b pure cholesterol material. HDL-C DCM employs direct precipitation of the apo-B-containing lipoproteins with dextran sulfate of 50 kDa with magnesium, followed by measurement using the reference method for TC (22).

Comparison protocol for TC

Those laboratories standardizing TC methods followed the CRMLN's certification protocol for clinical laboratories. The protocol required the laboratory to collect a set of six fresh individual serum specimens, however they could combine serum up to two individual donors to obtain the necessary volume or concentrations. The following guidelines for collection were provided to the laboratories: 1) Collect two samples in each of three concentration regions: 2.59–5.17 mmol/L (100–200 mg/dL), 5.17–6.21 mmol/L (200–240 mg/dL), and > 6.21 mmol/L (> 240 mg/dL); 2) make sure the range of concentration between the lowest and the highest is at least 2.59 mmol/L (100 mg/dL); and 3) make sure at least 0.52 mmol/L (20 mg/dL) difference exist between the concentrations of samples in each of the three regions.

All participants used commercially prepared enzymatic reagents and human serum-based calibrators. The assay principle of all reagents is the cholesterol ester hydrolase-cholesterol oxidase-peroxidase chromogenic method. Specimens were analyzed in duplicate on three separate days for a total of six replicate measurements per sample. After these measurements were completed, frozen aliquots were shipped on dry ice by overnight express delivery to OMC.

Selection of laboratories for TC

Of the 291 Japanese clinical laboratories, 78 laboratories were selected for this study because they participated two or more times. Some of these laboratories were involved in an epidemiological study for the Japan Public Health Center-based prospective Study on cancer and cardiovascular diseases (JPHC Study) (27) and a clinical trial for the Pravastatin Anti-atherosclerosis Trial in the Elderly (PATE) (12, 28).

Initially, we compared the results of clinical laboratories that participated twice, and subsequently, we compared the results of laboratories that participated more

than twice during a 5-year period. During the 5 years of the study, 10 standardization rounds were conducted. Of the 78 laboratories that participated in the first two rounds, the number declined over 5 years so that only 9 of the original laboratories remained in the 10th round.

Data analysis for TC

The analysis spreadsheet calculated average percentage bias, average absolute percentage bias, average within-sample CV, within- and between-method outliers, and linear regression statistics. The regression statistics were used to calculate the bias at the medical decision points of 5.17 and 6.21 mmol/L (200 and 240 mg/dL). Laboratories meeting the following criteria were qualified to receive "Certificate of Traceability": average absolute percentage bias $\leq 3\%$, percentage bias at 5.17 and 6.21 mmol/L $\leq 3\%$, CV $\leq 3\%$, correlation coefficient (r^2) ≥ 0.975 , and no within- or between-method outliers. The certificate is valid for 6 months.

Comparison protocol for HDL-C

Those laboratories standardizing HDL-C methods followed the CRMLN's evaluation protocol for manufacturers (26). The sample comparison is based upon the US National Committee for Clinical Laboratory Standards protocol "Method comparison and bias estimation using patient samples; approved guideline" (29). The protocol requires analysis of a minimum of 40–50 fresh patient specimens. Samples were selected with the range of HDL-C concentrations, 0.52–1.81 mmol/L (20–70 mg/dL). To achieve this goal, a minimum of five samples were collected in each of the following concentration regions: 0.52–0.75 mmol/L (20–29 mg/dL), 0.78–1.01 mmol/L (30–39 mg/dL), 1.03–1.27 mmol/L (40–49 mg/dL), 1.29–1.53 mmol/L (50–59 mg/dL), and 1.55–1.78 mmol/L (60–69 mg/dL). The remaining samples, a minimum of 15, were spread over the entire concentration range. All samples had triglyceride concentration < 2.26 mmol/L (200 mg/dL).

All participants used commercially prepared reagent kits and human serum-based calibrators. All of these methods are the "direct" methods. Not all laboratories used the same kit, but the products used were from three Japanese manufacturers: Kyowa Medex Co., Ltd., Tokyo (30); Daiichi Pure Chemicals Co., Ltd., Tokyo (31); and Wako Pure Chemical Industries, Ltd., Osaka (32).

The fresh-frozen serum samples were prepared at OMC from fasting donors including patients and volunteers. Serum was dispensed into separate vials and frozen at -60°C or below within 8 hours after separation. The frozen samples after check of lipoprotein electrophoresis were shipped to each participant on dry ice by overnight express delivery within 3 days of sample collection.

Clinical laboratories analyzed each sample in duplicate in one run. The total number of samples was divided

among five analytical runs. Between analytical runs the samples were stored at -60°C or below. Each laboratory analyzed its own quality control (QC) sample with HDL-C concentration of 0.78 to 1.55 mmol/L (30 to 60 mg/dL). Each laboratory used either a commercial QC product or prepared its own sample from pooled human serum. Single measurements from 20 recent analytical runs, including the runs where comparison samples were analyzed, were used to estimate among-run CV.

Selection of laboratories for HDL-C

Of the 200 Japanese clinical laboratories, 46 laboratories were selected for this study because they participated twice.

Data analysis for HDL-C

The analysis spreadsheet calculated average percentage bias, average absolute percentage bias, average within-sample within-run CV, within- and between-method outliers, and linear regression statistics. The regression statistics were used to calculate the bias at the medical decision points of 0.91 and 1.55 mmol/L (35 and 60 mg/dL). Among-run CV was also calculated from the single QC measurements with the field method. All laboratories obtained "Document of Comparison" stating that the specific analytical system had been compared with the DCM for HDL-C and listing the specific statistical parameters observed for the system. The document was valid for 2 years. For this study, the laboratories meeting the following criteria were considered to be standardized: average percentage bias $\leq 5\%$, percentage bias at the medical decision points of 0.91 and 1.55 mmol/L $\leq 5\%$, among-run CV $\leq 4\%$, $r^2 \geq 0.975$, no more than one within-method outlier, and no between-method outliers.

Statistics

For every survey and each laboratory (e.g., first round versus second round), we calculated mean percentage bias, mean absolute percentage bias, and CV. To compare overall group mean biases and CVs on the initial and second rounds, we used the student's *t*-test. We also calculated a *t*-statistic and *p*-value for each laboratory separately and evaluated these 78 *p*-values (33). A significance level of $\alpha = 0.05$ was used throughout this study.

In addition, for TC, we tested for reduction of bias over 10 surveys. To do this, we performed a linear weighted regression for each laboratory where the number of surveys (first to 10th) in which a laboratory participated was the independent variable and the percentage bias was the dependent variable. Weights corresponded to the inverse variances of the percentage biases obtained for each survey. Cases where the intercept is negative and the slope is positive, or vice versa, generally indicate improvement in the bias for the laboratory as more surveys are completed. We

plotted the intercepts versus the slopes for all 78 laboratories in a scatter plot. This procedure has the advantage of using all available data, rather than comparing only the first to the last surveys, for example.

Results

Standardization of TC in clinical laboratories

The average time between the first and second rounds for the 78 participating laboratories was 13 months; the median time difference was 7 months.

The performance of 78 clinical laboratories participating in the first and second rounds is presented in Table 1. Seventy-one (91.0%) and 72 (92.3%) laboratories met the performance criteria and received “Certificate of Traceability” in the first and second rounds, respectively. Overall, the pass rate in the second round did not improve significantly from the initial round.

The mean percentage bias for all laboratories improved by 0.44% between the first and second rounds, which was not statistically significant at the 95% level, but it suggests an actual difference. The mean absolute percentage bias improved by 0.20%, which did not reach statistical significance too. The mean average within-sample CV for the group of laboratories did not change.

In another evaluation of the data, we calculated the mean difference for each laboratory and formed a *t*-value for each laboratory on the basis of five degrees of freedom (from six sample means). Thus, 78 *p*-values are obtained. The median *p*-value for 78 laboratories was 0.024. Sixty-three of 78 (80.8%) were < 0.05 and statistically significant at the 95% probability level. Eighty

Fig. 1. Mean % Bias Plots for TC. Bias plots of 78 Japanese clinical laboratories that participated in the first and second TC certification round. Mean percentage bias is plotted versus the TC concentration (mg/dL) as determined by the AK reference method at Osaka. Horizontal dotted lines mark the NCEP bias guidelines at $\pm 3\%$. Data for all participants is presented together in this one plot. The regression line was $y = 0.0010 \times - 1.1715$ ($n = 78$, $r = 0.0284$) for the initial round ($\times$) and $y = - 0.0018 \times - 0.0626$ ($n = 78$, $r = - 0.0538$) for the second round ($\circ$).

percent of the laboratories showed significant improvement in their bias.

The bias of all participants in the initial and second rounds is shown in Fig. 1. This plot includes the bias of all individual samples analyzed by all 78 participants. Fewer samples had bias greater than $\pm 3\%$ in the second round.

Table 1. Performance of participants in total cholesterol certification program

Round & <i>p</i> -value	Participants		Accuracy% bias		PrecisionCV
			Mean % bias (Mean $\pm$ SD)	Mean absolute % bias (Mean $\pm$ SD)	Mean within-sample CV (Mean $\pm$ SD)
Initial	All Labs	78	$-0.93 \pm 1.77\%$	$1.61 \pm 1.27\%$	$0.72 \pm 0.33\%$
	Certified ^a	71	$-0.56 \pm 1.31\%$	$1.30 \pm 0.72\%$	$0.70 \pm 0.34\%$
Second	All Labs	78	$-0.49 \pm 1.75\%$	$1.41 \pm 1.20\%$	$0.69 \pm 0.30\%$
	Certified ^a	72	$-0.24 \pm 1.22\%$	$1.14 \pm 0.60\%$	$0.69 \pm 0.30\%$
<i>p</i> -value ^b	All Labs	78	0.11	0.31	0.65
	Certified		0.13	0.15	0.79

^a: Only laboratories that met CRMLN performance criteria: Average percentage bias $< \pm 3\%$, percentage bias at 5.17 and 6.21 mmol/L $< \pm 3\%$, CV $< 3\%$, correlation coefficient (r^2) < 0.975 , and no within- or between-method outliers.

^b: *p*-value for comparison of initial and second rounds

Table 2. Number of participants failing to meet specific CRMLN performance criteria for total cholesterol

Round	Accuracy	Precision	Outliers	
	Mean absolute% bias $\pm 3\%$	Mean within -sample CV $< 3\%$	Within-method outliers	Between-method outliers
Initial	7	0	1	0
Second	5	0	1	0

In the first round of standardization, a total of seven laboratories failed to meet the criteria to obtain “Certificate of Traceability”. In the second round, a total of five laboratories failed. Table 2 summarizes the reasons that laboratories did not pass certification for both rounds of the protocol. No laboratories failed certification because of imprecision. The most common reason for failure was inaccuracy. Some laboratories failed on multiple criteria. Only one laboratory failed both rounds; this laboratory failed the first time because of inaccuracy and the second time because of within-method outliers. In a third round, this laboratory met all of the criteria.

Table 3 shows the results of 10 rounds of the TC certification protocol with the 78 laboratories that began with the initial standardization. The number of laboratories that remained in the program for all 10 rounds decreased over the 5 years of the study. However, the pass rate increased by the sixth round. Although the mean percentage bias did not appear to change significantly, the mean absolute percentage bias was reduced over time. A weighed regression of the mean absolute bias over

the course of the 10 rounds (mean absolute percentage bias versus the number of surveys) had a slope of -0.050, an intercept of 1.340, and a *p*-value of 0.0008. This shows that the mean absolute percentage bias has been reduced significantly over the 10 surveys for these laboratories.

Standardization of HDL-C in clinical laboratories

The average period between the first and second events in the 46 participants was 20 months.

The initial and second performances of HDL-C by all participants is presented in Table 4. As a group, these laboratories' performance improved between the first and second events. For mean percentage bias and mean absolute percentage bias, differences between the initial and second events were statistically significant (*p* = 0.003 and *p* = 0.00002, respectively). Differences in the mean among-run CV between the first and second events were not statistically significant (*p* = 0.88). The percentage bias of all participants is shown in Fig. 2A and Fig. 2B for the first and second events, respectively.

Table 3. Trends in total cholesterol performance of certified laboratories

Round	Participants # (remaining labs (%))	Pass rate (%)	Accuracy % bias		Precision CV
			Mean % bias (Mean ± SD)	Mean absolute % bias (Mean ± SD)	Mean within-sample CV (Mean ± SD)
1	78	91.00%	-0.56 ± 1.31%	1.30 ± 0.72%	0.70 ± 0.34%
2	78 (100.0%)	92.30%	-0.24 ± 1.22%	1.14 ± 0.60%	0.69 ± 0.30%
3	47 (60.3%)	100.00%	0.00 ± 1.28%	1.20 ± 0.59%	0.63 ± 0.30%
4	36 (46.2%)	94.40%	-0.22 ± 1.25%	1.09 ± 0.69%	0.67 ± 0.34%
5	34 (43.6%)	91.20%	0.07 ± 1.34%	1.23 ± 0.60%	0.61 ± 0.27%
6	29 (37.2%)	100.00%	-0.11 ± 1.15%	1.04 ± 0.54%	0.64 ± 0.25%
7	26 (33.3%)	100.00%	-0.02 ± 1.27%	1.12 ± 0.65%	0.63 ± 0.27%
8	22 (28.2%)	95.50%	-0.28 ± 0.89%	0.90 ± 0.43%	0.67 ± 0.23%
9	18 (23.1%)	100.00%	-0.26 ± 1.05%	0.93 ± 0.59%	0.61 ± 0.35%
10	9 (11.5%)	100.00%	-0.29 ± 0.78%	0.66 ± 0.51%	0.68 ± 0.29%

Table 4. Performance of participants in HDL-cholesterol evaluation program

Event and <i>p</i> -value	Accuracy % bias		Precision CV
	Mean % bias (Mean ± SD)	Mean absolute % bias (Mean ± SD)	Among-run CV (Mean ± SD)
Initial	-1.86 ± 3.01%	4.22 ± 1.52%	1.56 ± 0.76%
Second	-0.06 ± 2.71%	2.88 ± 1.29%	1.58 ± 0.77%
<i>p</i> -value ^a	0.003	0.00002	0.88

^a: *p*-value for comparison of initial and second events.

Table 5. Number of participants failing to meet specific CRMLN performance criteria for HDL-cholesterol

Event	Accuracy	Precision	Outliers	
	Absolute % bias ± 5%	Run CV < = 4%	Within-method outliers	Between-method outliers
Initial	16	0	2	0
Second	2	1	3	0

Fig. 2. Mean % Bias Plots for HDL-C. Bias plots of 46 Japanese clinical laboratories that participated in two evaluations of HDL-C performance. Mean percentage bias is plotted versus the HDL-C concentration (mg/dL) as determined by the designated comparison method at Osaka. Horizontal dotted lines mark the NCEP bias guidelines at $\pm 5\%$. Data for all participants is presented together in each plot. Figure 2 A: The regression line was $y = -0.1295x + 4.2998$ ($n = 46$, $r = -0.4549$) for the initial event ($\times$). Figure 2 B: The regression line was $y = -0.0324x + 1.4207$ ($n = 46$, $r = -0.1380$) for the second event ($\circ$).

Table 5 summarizes the problems that some of these laboratories had in meeting the performance criteria. Precision was not a common problem. However, a number of laboratories had accuracy problems, particularly in the first event. The accuracy problems improved considerably in the second event of evaluations.

Discussion

Achieving accuracy within a laboratory and comparability between laboratories requires traceability to a defined common accuracy base. The most practical approach to achieve traceability is to ensure that manufacturers properly calibrate diagnostic products. This has been the primary focus of the CRMLN since its inception. Another approach to achieve comparability is to require that all clinical laboratories use the same method; however, this is neither practical or possible.

In Japan, no homogeneous systems exist where instrument, calibrators, and reagents are marketed by a single manufacturer. More commonly, heterogeneous systems are used where an instrument is purchased from one manufacturer, and calibrators and reagents are purchased from another manufacturer. A laboratory using a heterogeneous analytical system must assume primary responsibility for documenting performance and establishing traceability to the accuracy base. For this reason, many Japanese laboratories have chosen to participate in the CRMLN traceability programs. This offered us a unique opportunity to evaluate the impact of TC standardization and HDL-C evaluation by comparison of the initial and subsequent performances by clinical laboratories using all heterogeneous analytical systems.

Japanese manufacturers set accurate values on calibrators in three ways: 1) by performing a fresh sample comparison with OMC; this approach has been used for both TC and HDL-C; 2) by sending the calibrator to OMC for value-assignment; this approach has been used for HDL-C; and 3) OMC certifying enzymatic methods through the manufacturer's certification protocol; the manufacturers then use the enzymatic methods to assign calibrator values in-house.

For TC, accuracy by the certified laboratories tended to improve in the second standardization compared to the initial standardization, however the improvement was not statistically significant. Although the mean bias for all laboratories was reduced by half between the initial and second performances, the difference is not statistically significant because of the relatively high variation between laboratories for this parameter. The p -value (0.11) observed for the t -test of the mean performance for the entire group of laboratories was not significant at the 95% level, but suggested a real difference. Although bias still exists, the smaller bias than in the initial standardization indicates that the laboratories improved their accuracy, namely the change in bias was in the desirable direction. The variability used to test this reflects among-laboratory variation that reduces the likelihood of finding a significant result.

The statistical tests to evaluate individual laboratories' performance between the first and second rounds confirm the suggestion of improvement in performance. This second approach avoids integrating the among-laboratory variability and is more powerful and confirms a trend for laboratories to improve between the first and second rounds.

The mean overall precision for this group of laboratories stayed very nearly the same between the initial and second performances. This is consistent with the fact that precision is not a problem with TC measurements. In fact, none of the laboratories failed to be certified because they did not meet the precision criterion.

When a laboratory failed to meet the criteria, OMC consulted with the laboratory to assist in determining the sources of and resolving the problem. The consultation would consist of a telephone call and/or a visit to OMC from laboratory personnel. If the source of the problem was determined to be with the calibrators or reagents, OMC consulted with the manufacturer to assist in resolving the problem. After the source of the problem was resolved, the laboratory had the opportunity to immediately participate in the certification protocol again. This consultation and certification procedure was followed until the laboratory could verify that it met the performance criteria.

Accuracy failure occurred in 11 laboratories during the first two rounds. The accuracy problem was resolved by changing the calibrator lot (two laboratories), changing of calibrator supply source (five laboratories), or stabilizing an unstable instrument (one laboratory). However, the cause in inaccuracy was not resolved in three laboratories.

The mean absolute percentage bias had a significant trend to lower values as laboratories continued participation in the certification program. We believe that this emphasizes the importance of regular participation with six months interval for TC over at least three years. We observed that, in general, laboratories that met the certification criteria in the first round continued to improve their bias the longer they participated in the program.

For HDL-C, accuracy was significantly improved in the second evaluation over the initial evaluation (34). Precision did not markedly improve. In the HDL-C evaluations, precision failure was resolved by maintenance of an unstable instrument. The accuracy problems that occurred in nine laboratories were resolved by reconstitution of the calibrator in five laboratories and a change of calibrator lot in three laboratories. The cause of inaccuracy was not resolved in one laboratory. Failure because of within-method outliers was resolved by readjustment of an unstable instrument in three laboratories and by a change in technologist in one laboratory. The cause of within-method outliers remained unresolved in one laboratory.

We understand that it will be desirable for using fresh-non-frozen samples for HDL-C measurement. However, in this study the fresh-frozen serum samples stocked at -60°C or below were used because first the HDL-C measurement should be divided among five analytical runs, namely five weeks as one run in a week and because second the reports are available for HDL-C can be determined accurately after storage at -70°C for up 1 month

to 1 or 2 years (19,35). Any change in the samples was not found in the check of lipoprotein electrophoresis.

Commercial available kits for HDL-C measurement are developed based on various methodological principles (34). For this reasons the differences sometimes produce serious discrepancy among them for the patient samples that may have specific lipoprotein abnormality. This problem has not clearly been sorted and reported. Therefore, the manufacturers reagent kits should be furthermore focused to improvement for measurement of patient samples with lipid disorders.

In conclusion, accuracy for TC tended to improvement although not significantly, but for HDL-C improved between the initial and subsequent events. Precision was not significantly changed for either TC or HDL-C between the initial and subsequent rounds. Sustained participation in the TC certification program for 5 years demonstrated improved performance the longer a laboratory remained in the program, even while meeting the CRMLN performance criteria. We believe that continuous participation in the international standardization program from every clinical laboratory in Japan is a very essential part not only of the clinical or epidemiological study and practice for the risk management treatment but also of overseas publication of results in medical research involving Japanese peoples. The results of this study demonstrate that, at the outset of participation in the certification program, inaccuracy in TC and HDL-C testing was more of a problem than imprecision. CRMLN certification protocols will contribute effectively to improved accuracy for TC and HDL-C measurements.

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References

- (1) Gordon T, Kannel WB, Castelli WP, and Dawber TR: Lipoproteins, cardiovascular disease, and death: The Framingham study. *Arch Intern Med*, 141: 1128-1131, 1981
- (2) Lipid Research Clinics Program: The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease.

- II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA*, 251: 351–374, 1984
- (3) Stamler J, Wentworth D, and Neaton JD, for the MRFIT Research Group: Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA*, 256: 2823–2828, 1986
- (4) The Kyushu Lipid Intervention Study Group: A coronary primary intervention study of Japanese men: study design, implementation and baseline data. *J Atheroscler Thromb*, 3: 95–104, 1996
- (5) Yamamoto A, Richie G, Nakamura H, Hosoda S, Nobuyoshi M, Matsuzaki M, Tan CE, and Keech A for the ASPAC study members in Japan, and Mabuchi H, Horibe H, and Tenba H for the Research Committee on Serum Lipid Survey 1990 Japan: Risk factors for coronary heart disease in the Japanese—Comparison of the background of patients with acute coronary syndrome in the ASPAC study with data obtained from the general population. *J Atheroscler Thromb*, 9: 191–199, 2002
- (6) Gordon DJ and Rifkind BM: High density lipoprotein: the clinical implications of recent studies. *N Engl J Med*, 321: 1311–1316, 1989
- (7) Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease: Triglyceride, high-density lipoprotein, and coronary heart disease. *JAMA*, 269: 505–510, 1993
- (8) Kitamura A, Iso H, Naito Y, Iida M, Konishi M, Folsom AR, *et al*: High-density lipoprotein cholesterol and premature coronary heart disease in urban Japanese men. *Circulation*, 89: 2533–2539, 1994
- (9) Scandinavian Simvastatin Survival Group: Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, 344: 1383–1389, 1994
- (10) Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, *et al*: Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med*, 333: 1301–1307, 1995
- (11) The Kyushu Lipid Intervention Study Group: Pravastatin use and risk of coronary events and cerebral infarction in Japanese men with moderate hypercholesterolemia. *J Atheroscler Thromb*, 7: 110–121, 2000
- (12) Ito H, Ouchi Y, Ohashi Y, Saito Y, Ishikawa T, Nakamura H, and Orimo H: A comparison of low versus standard dose pravastatin therapy for the prevention of cardiovascular events in the elderly: the Pravastatin Anti-atherosclerosis Trial in the Elderly (PATE). *J Atheroscler Thromb*, 8: 33–44, 2001
- (13) Saito Y, Shirai K, Sasaki N, Shinomiya M, and Yoshida S for the Committee of the Chiba Lipid Intervention Program (CLIP) Study: Prognosis of hypercholesterolemic patients taking pravastatin for five years. *J Atheroscler Thromb*, 9: 99–108, 2002
- (14) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, 285: 2486–2497, 2001
- (15) Prevention of coronary heart disease in clinical practice. Recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. *Eur Heart J*, 19: 1434–1503, 1998
- (16) Japan Atherosclerosis Society (JAS) guidelines for diagnosis and treatment of atherosclerotic cardiovascular diseases, 2002
- (17) Recommendations for Improving Cholesterol Measurement. A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program. NIH Publication No.93–2964. Bethesda, MD: National Heart, Lung, and Blood Institute, 1993
- (18) National Cholesterol Education Program. Working Group on Lipoprotein Measurement. Recommendations on lipoprotein measurement. NIH publication No.95–3044. Bethesda, MD: National Heart, Lung, and Blood Institute, 1995
- (19) Warnick GR and Wood PD for the National Cholesterol Education Program Working Group on Lipoprotein Measurement: National Cholesterol Education Program recommendations for measurement of high-density lipoprotein cholesterol: Executive Summary. *Clin Chem*, 41: 1427–1433, 1995
- (20) McNamara JR, Leary ET, Ceriotti F, Cobbaert CM, Cole TG, Hassemer DJ, *et al*: Status of lipid and lipoprotein standardization. *Clin Chem*, 43: 1306–1310, 1997
- (21) Myers GL, Kimberly MM, Waymack PP, Smith SJ, Cooper GR, and Sampson EJ: A reference method laboratory network for cholesterol: A model for standardization and improvement of clinical laboratory measurements. *Clin Chem*, 46: 1762–1772, 2000
- (22) Kimberly MM, Leary ET, Cole TG, and Waymack PP for the Cholesterol Reference Method Laboratory Network: Selection, validation, standardization, and performance of a designated comparison method for HDL-cholesterol for use in the Cholesterol Reference Method Laboratory Network. *Clin Chem* 45: 1803–1812, 1999
- (23) Abell LL, Levy BB, Brodie BB, and Kendall FE: A simplified method for the estimation of total cholesterol in serum and demonstration of its specific-

- ity. *J Biol Chem*, 195: 357-366, 1952
- (24) Cooper GR, Myers GL, Smith SJ, and Sampson EJ: Standardization of lipid, lipoprotein, and apolipoprotein measurements. *Clin Chem*, 34: B95-B105, 1988
- (25) Certification Protocol for Clinical Laboratories, Cholesterol Reference Method Laboratory Network and the Centers for Disease Control and Prevention, June 1994
- (26) HDL Cholesterol Method Certification Protocol for Manufacturers, Cholesterol Reference Method Laboratory Network and the Centers for Disease Control and Prevention, June 1999
- (27) Iida M, Sato S and Nakamura M for the JPHC Study Group: Standardization of laboratory test in the JPHC study. *J Epidemiol*, 11(Suppl): S81-S86, 2001
- (28) Nakamura M, Iida M, Orimo H, and Nakamura H: Standardization of serum total cholesterol by CDC/CRMLN protocol. *Domyaku Koka*, 27:7-15, 2000 (in Japanese)
- (29) National Committee for Clinical Laboratory Standards: Method comparison and bias estimation using patient samples; approved guideline. NCCLS Document EP9-A. Villanova, PA: NCCLS, 1995
- (30) Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, *et al*: Direct measurement of high-density-lipoprotein cholesterol in serum with polyethylene-glycol-modified enzymes and sulfated alpha-cyclodextrin. *Clin Chem*, 41: 717-723, 1995
- (31) Hubbard RS, Hirany SV, Devaraj S, Martin L, Parupia J, and Jialal I: Evaluation of a rapid homogeneous method for direct measurement of high-density lipoprotein cholesterol. *Am J Clin Pathol*, 110: 495-502, 1998
- (32) Miki Y, Hatanaka S, Hanada T, Matsuura S, and Malloy T: A homogeneous assay for the selective measurement of HDL-cholesterol in serum [abstract]. *Clin Chem*, 43: S256, 1997
- (33) Westgard JO and Barry PL: Cost-effective quality control: Managing the quality and productivity of analytical process, AACC 1995
- (34) Nakamura M, Sato S, and Iida M: Performance evaluation of homogeneous HDL-cholesterol assays in 48 Japanese clinical laboratories [abstr]. *Clin Chem*, 43: S259-S260, 1997
- (35) Bausserman LL, Saritelli AL, and Milosavljevic D: High-density lipoprotein subfractions measured in stored serum. *Clin Chem*, 40: 1713-1716, 1994