[32] Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 2010;56:977–86.
[33] Miida T, Nishimura K, Okamura T, et al. A multicenter study on the precision and accuracy of homogeneous assays for LDL-cholesterol: comparison with a beta-quantification method using fresh serum obtained from non-diseased and diseased subjects. Atherosclerosis 2012;225:208–15.
[34] Cole TG, Contois JH, Csako G, et al. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC lipoprotein and vascular diseases division working group on best practices. Clin Chem 2013;59:752–70.
[35] Contois JH, Warnick GR, Sniderman AD. Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B measurement. J Clin Lipid 2011;5:264–72.
[36] Warnick GR. Lack of agreement of homogeneous assays with the reference method for LDL-cholesterol may not indicate unreliable prediction of risk for cardiovascular disease. Clin Chem 2002;48:1812–5.
[37] AACC Lipoproteins and Vascular Diseases Division Working Group on Best PracticesCole TG, Contois JH, et al. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices. Clin Chem 2013;59:752–70.

Clinica Chimica Acta 439 (2015) 185–190

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

HDL cholesterol performance using an ultracentrifugation reference measurement procedure and the designated comparison method☆

Masakazu Nakamura [a,*], Shinji Yokoyama [b], Yuzo Kayamori [c], Hiroyasu Iso [d], Akihiko Kitamura [d], Tomonori Okamura [e], Masahiko Kiyama [f], Hiroyuki Noda [d,g], Kunihiro Nishimura [h], Michikazu Nakai [i], Isao Koyama [a], Mahnaz Dasti [j], Hubert W. Vesper [j], Tamio Teramoto [k], Yoshihiro Miyamoto [l]

[a] National Cerebral and Cardiovascular Center, Department of Preventive Cardiology, Lipid Reference Laboratory, Japan
[b] Nutritional Health Science Research Center, Chubu University, Japan
[c] Department of Health Sciences, Faculty of Medical Sciences, Kyushu University, Japan
[d] Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Japan
[e] Department of Preventive Medicine and Public Health, Keio University, Japan
[f] Osaka Center for Cancer and Cardiovascular Disease Prevention, Japan
[g] Cancer Control and Health Promotion Division, Health Service Bureau, Ministry of Health, Labour and Welfare, Japan
[h] National Cerebral and Cardiovascular Center, Department of Preventive Medicine and Epidemiologic Informatics, Office of Evidence-based Medicine and Risk Analysis, Japan
[i] National Cerebral and Cardiovascular Center, Department of Preventive Medicine and Epidemiologic Informatics, Japan
[j] Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, USA
[k] Teikyo Academic Research Center, Teikyo University, Japan
[l] National Cerebral and Cardiovascular Center, Department of Preventive Cardiology, Department of Preventive Medicine and Epidemiologic Informatics, Japan

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 6 October 2014
Accepted 27 October 2014
Available online 31 October 2014

Keywords:
HDL cholesterol
Ultracentrifugation
Designated comparison method
CDC
CRMLN

ABSTRACT

Background: Accurate high-density lipoprotein cholesterol (HDL-C) measurements are important for management of cardiovascular diseases. The US Centers for Disease Control and Prevention (CDC) and Cholesterol Reference Method Laboratory Network (CRMLN) perform ultracentrifugation (UC) reference measurement procedure (RMP) to value assign HDL-C. Japanese CRMLN laboratory (Osaka) concurrently runs UC procedure and the designated comparison method (DCM). Osaka performance of UC and DCM was examined and compared with CDC RMP.

Methods: CDC RMP involved UC, heparin-$MnCl_2$ precipitation, and cholesterol analysis. CRMLN DCM for samples containing <200 mg/dl triglycerides involved 50-kDa dextran sulfate-$MgCl_2$ precipitation and cholesterol determination.

Results: HDL-C regression equations obtained with CDC (x) and Osaka (y) were $y = 0.992x + 0.542$ ($R^2 = 0.996$) for Osaka UC and $y = 1.004x - 0.181$ ($R^2 = 0.998$) for DCM. Pass rates within ±1 mg/dl of the CDC target value were 91.9 and 92.1% for Osaka UC and DCM, respectively. Biases at 40 mg/dl HDL-C were +0.22 and −0.02 mg/dl for Osaka UC and DCM, respectively.

Conclusions: Osaka UC and DCM were highly accurate, precise, and stable for many years, assisting manufacturers to calibrate products for clinical laboratories to accurately measure HDL-C for patients, calculate non-HDL-C, and estimate low-density lipoprotein cholesterol with the Friedewald equation.

1. Introduction

A low high-density lipoprotein cholesterol (HDL-C) level is a strong biomarker for predicting the risk of cardiovascular diseases (CVD), as demonstrated by several epidemiological studies and clinical trials [1–3]. The US National Cholesterol Education Program (NCEP) estimated that each 1% increase in HDL-C may be associated with a 2–4% decrease in the risk of coronary heart disease (CHD), and clinical trials on low-density lipoprotein-lowering therapies have shown that concomitant increases in HDL-C confer an additional independent reduction in the risk of CHD [4]. HDL-C together with low-density lipoprotein cholesterol (LDL-C), total cholesterol, and triglycerides form a lipid panel that is measured in routine patient care to determine and monitor the risk of a patient developing CVD.

Accurate and reproducible HDL-C measurements are of particular importance for correctly and consistently classifying individuals at risk of CVD, as outlined in the clinical guidelines for the subsequent

☆ Disclaimer: The results and conclusions in this study are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

* Corresponding author at: National Cerebral and Cardiovascular Center, Department of Preventive Cardiology, Lipid Reference Laboratory, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5004; fax: +81 6 6833 5300.
E-mail address: nakamura.masakazu.hp@ncvc.go.jp (M. Nakamura).

http://dx.doi.org/10.1016/j.cca.2014.10.039
0009-8981/

diagnosis, treatment, and prevention of patients [5–7]. Furthermore, the US NCEP reported [4] that the accuracy of HDL-C was particularly important because (a) the inverse association of HDL-C with the risk of CHD is expressed over a relatively narrow concentration range, (b) the medical decision cut-off point (40 mg/dl) for an increased risk of CHD is at the lower end of the HDL-C concentration range, at which small errors can have a strong impact on patient classification, and (c) the calculation of non-HDL-C [8,9] or LDL-C using the Friedewald equation [10]. Inaccurate HDL-C measurements also lead to errors in the estimation of LDL-C.

Previous studies recommended that the US Centers for Disease Control and Prevention (CDC) reference measurement procedure (RMP) should be used to achieve accurate HDL-C measurements. CDC RMP is a three-step procedure [11,12]: (1) ultracentrifugation (UC) at d = 1.006 kg/l to remove triglyceride-rich lipoproteins; (2) precipitation of apo B-containing lipoproteins from the ultracentrifugal infranatant with heparin-$MnCl_2$; (3) measurement of cholesterol in the heparin-$MnCl_2$ supernatant using the CDC reference method for cholesterol [13]. However, ultracentrifugal measurements of HDL-C have low sample throughput and require equipment that is not commonly available in routine clinical laboratories. Therefore, the Cholesterol Reference Method Laboratory Network (CRMLN) sought to implement a designated comparison method (DCM) [14–16] with the objective of better assisting reagent manufacturers in the calibration of their products so that clinical laboratories could more accurately measure HDL-C for patients, calculate non-HDL-C, and estimate LDL-C with the Friedewald equation.

The CDC UC method has been accepted as the most reliable RMP for HDL-C and the CRMLN DCM is an accurate, robust, transferable and practical method for clinical laboratories and manufacturers. As part of the CRMLN activities, the National Cerebral and Cardiovascular Center at Osaka, Japan has implemented and maintained 1) the UC method, which is same as CDC RMP, for 17 years since May 1997 and 2) DCM for 20 years since April 1994. We measured the performance of both HDL-C reference methods in terms of accuracy and reproducibility after many years using comparisons with CDC RMP.

2. Materials and methods

2.1. Materials

All standardization pools for HDL-C were prepared according to the Clinical Laboratory Standards Institute document C37-A (Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline), which implied that no preservatives or no additives were added. All survey pools were blinded to the CRMLN laboratories. They were shipped frozen from CDC and stored at −70 °C before analysis.

HDL-C assays were conducted in the Osaka Medical Center for Cancer and Cardiovascular Diseases between July 1997 and June 2001, in the Osaka Medical Center for Health Science and Promotion between July 2001 and March 2012, and in the National Cerebral and Cardiovascular Center at Osaka continuously from April 2012 (all laboratories were referred to as the 'Osaka' laboratory).

2.2. Methods

2.2.1. CDC reference measurement procedure for ultracentrifugation

The first step of CDC RMP employed preparative ultracentrifugation (Beckman Coulter, Optima L-70 K and/or Optima XE-90) to remove apo B-containing lipoproteins [11,12]. The methods at CDC and Osaka used 5.00 ml of serum at a density of d = 1.006 kg/l (0.195 mol/l NaCL solution) and a 50.4Ti rotor (Beckman Coulter). UC at CDC was carried out for 16.2 h at 120,000 ×g and 18 °C, and at Osaka for 18.5 h at 105,000 ×g and 18 °C. After UC, the top fraction (d < 1.006 kg/l) was removed using tube slicer and the bottom fraction (d > 1.006 kg/l) was quantitatively transferred to a 5.00 ml volumetric flask adjusting with 0.15 mol/l NaCl solution [14–16]. In the second step, 1.00 ml aliquots of the bottom fraction were precipitated with 40 μL heparin (sodium injection, 5000 USP units/ml, Baxter Healthcare Corporation) and 50 μL manganese reagents ($MnCl_2$ solution, 1.00 M ±0.01 M, SIGMA) [17]. The precipitate was removed for 30 min at 1500 ×g and 4 °C [18–20]. In the third step, HDL-C was determined in the supernatant in duplicate measurements by the Abell–Kendall reference method for cholesterol [13]. The recovered cholesterol value was multiplied by 1.09 to account for the dilution introduced by the addition of the precipitation reagent. Four replicates from each sample were used in comparisons of assay performance.

2.2.2. CRMLN designated comparison method

DCM is a precipitation-based designated comparison method using 50-kDa dextran sulfate (DS)-$MgCl_2$ as the reagent. DS (stored at 2 to 8 °C. Kept tightly capped in a desiccator in a refrigerator after opening) was obtained from Warnick & Co. and was a special lot (lot#: 162176) for CRMLN use only. All CRMLN laboratories used the same DS lot to minimize potential lot-to-lot variations. $MgCl_2 \cdot 6H_2O$ (this reagent was highly hygroscopic and had to be dried. A larger amount than was needed was placed in a beaker and dried in an oven at 37 °C for at least one hour) and sodium azide (NaN_3) were obtained from Wako Pure Chemistries Inc. in Japan. The stock solution of DS contained 2.0 g/dl DS including 50 mg/dl NaN_3, while that of $MgCl_2$ contained 14.22 g/dl including 50 mg/dl NaN_3. The working reagent was prepared by mixing equal volumes. The working solution was stored at 2 and 8 °C [15,16]. Osaka laboratory previously confirmed that it was stable for 3 years.

In the first step, the samples and working reagent were equilibrated to room temperature and mixed at a ratio of 1.00 ml specimen and 0.10 ml working reagent. The samples for DCM required normotriglyceridemic sera including <200 mg/dl in triglycerides because of its limited sedimentation efficiency [16,17]. The samples were then incubated at room temperature for 10–30 min and centrifuged for 30 min at 4 °C at 1500 ×g. In the second step, clear supernatants were analyzed using the reference method for cholesterol [13]. The recovered HDL-cholesterol value in the DCM was multiplied by 1.1. HDL-C was assayed in the supernatant in duplicate measurements. Four replicates from one aliquot were used in comparisons of assay performance.

2.3. Performance criteria for HDL-C applied to CRMLN laboratories

The performance criteria for HDL-C applied to the CRMLN lipid reference laboratories are summarized in Table 1. Imprecision is evaluated not in coefficient variation (CV), but in standard deviation (SD, unit: mg/dl), and accuracy is evaluated in bias (mg/dl) from CDC reference value.

2.4. Statistical analysis

We used protocol EP9-A from the Clinical and Laboratory Standards Institute for bias estimation [21] and the STATA12 analysis program for all other calculations.

Table 1
Performance criteria applied to CRMLN lipid reference laboratory using UC method and DCM for HDL-C.

Lipid	Imprecision criterion	Accuracy criterion
HDL-C	Standard deviation ≤1 mg/dl	Bias ≤1 mg/dl

CRMLN: cholesterol reference method laboratory network. UC: ultracentrifugation. DCM: designated comparison method. HDL-C: high-density lipoprotein cholesterol.

3. Results

3.1. Accuracy

In the UC procedure at Osaka, the pooled serum with 160 different concentrations (lots) for HDL-C were analyzed among 626 survey samples with 154 survey runs, in which each survey run consisted of 3 to 5 different pools. They were analyzed for 17 years between May 1997 and January 2014. The concentration ranges were 26.9–78.9 mg/dl. In the scatter plots of bias (unit: mg/dl) between Osaka (y) and CDC (x), $y = -0.008x + 0.540$ ($R^2 = 0.017$). The p-values and 95% confidence interval (CI) of the slopes and intercepts were 0.001 and (−0.013, −0.003), and <0.001 and (0.296, 0.784), respectively (Table 2). The Osaka laboratory met acceptable accuracy goals for 91.9% (575 of 626 samples) within ±1 mg/dl of the CDC reference values (Fig. 1A). Biases between the target values of CDC and the measurements of Osaka at two medical decision points of 40 and 60 mg/dl were 0.22 and 0.06 mg/dl, respectively, both of which were slightly on the positive side. Although the bias and SD scattering of DCM appeared to be slightly better than that of CDC RMP, no significant differences (p-value: 0.05) were observed in the accuracy or precision of the 2 procedures.

In the DCM at Osaka, the pooled serum with 163 different concentrations (lots) for HDL-C were analyzed among 570 survey samples with 147 survey runs, in which each survey run consisted of 3 to 4 different pools. They were analyzed for 20 years between April 1994 and January 2014. The concentration ranges were 20.8–86.0 mg/dl. In the scatter plots of bias (unit: mg/dl) between Osaka (y) and CDC (x), $y = 0.004x - 0.181$ ($R^2 = 0.006$). The p-values and 95% CI of the slopes and intercepts were 0.065 and (−0.0002, 0.007), and 0.062 and (−0.370, 0.009), respectively (Table 2). The Osaka laboratory met acceptable accuracy goals for 92.1% (525 of 570 samples) within ±1 mg/dl of the CDC reference values (Fig. 1B). Biases between the target values of CDC and measurements of Osaka at two medical decision points of 40 and 60 mg/dl were −0.02 and +0.06 mg/dl, respectively, both of which were slightly biased.

3.2. Precision

In the scatter plots of SD between Osaka (y) and CDC (x), y (SD, mg/dl) = 0.002x (CDC reference value) + 0.270 [n: 626, $R^2 = 0.006$]. The p-value and 95% CI for the slope were 0.056 and (−0.00005, 0.0036), respectively. The p-value and 95% CI for the intercept were <0.001 and (0.179, 0.360), respectively (Table 2). The Osaka laboratory met acceptable precision goals for 97.9% (613 of 626 samples) within ±1 mg/dl. The maximum SD at Osaka UC was 2.3 mg/dl (Fig. 2C).

In the scatter plots of SD between Osaka (y) and CDC (x), y (SD, mg/dl) = 0.001x (CDC reference value) + 0.218 [n: 570, $R^2 = 0.005$].

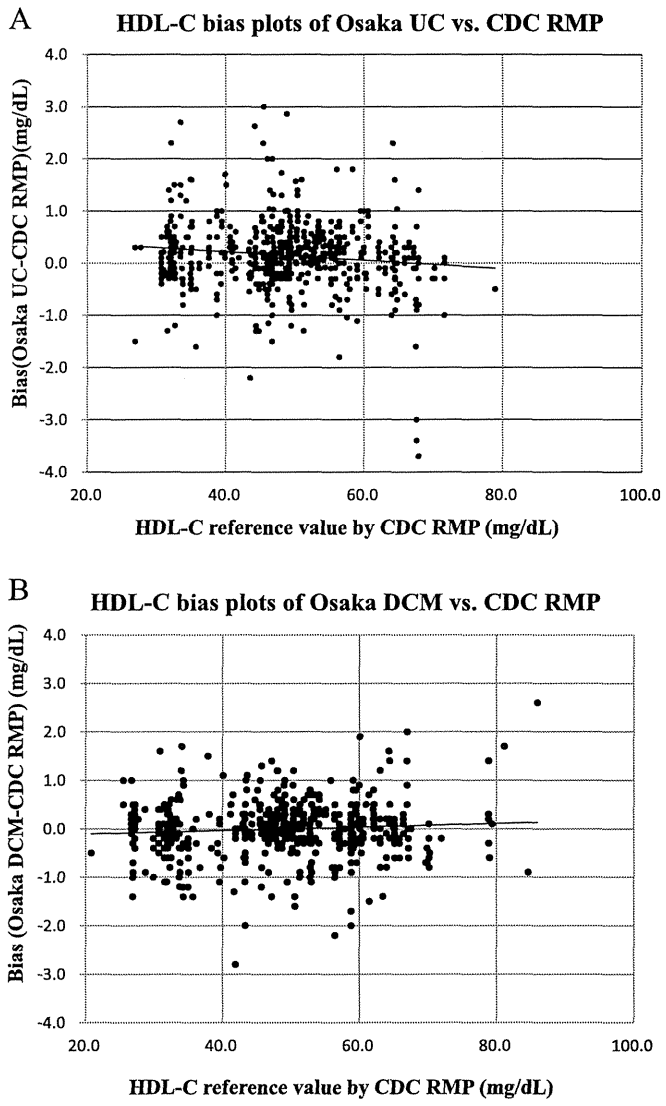

Fig. 1. A: HDL-C bias plots of Osaka UC vs. CDC RMP. The y-axis indicates the bias (mg/dl) of Osaka UC compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. UC: Ultracentrifugation. B: HDL-C bias plots of Osaka DCM vs. CDC RMP. The y-axis indicates the bias (mg/dl) of the Osaka DCM compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. DCM: Designated comparison method.

Table 2
Regression analysis of the bias between Osaka (y) and CDC (x) and imprecision for HDL-C over time (unit: mg/dl).

Parameter	HDL-C method	Number of samples	Slope (95%CI)	Intercept (95%CI)	R^2	Time period
Accuracy	UC	626	−0.008 (−0.013, −0.003) p = 0.001	0.540 (0.296, 0.784) p < 0.001	0.017	May 1997 to January 2014 (17 years)
	DCM	570	0.004 (−0.0002, 0.07) p = NS	−0.181 (−0.370, 0.009) p = NS	0.006	April 1994 to January 2014 (20 years)
Precision	UC	626	0.002 (−0.00005, 0.0036) p = NS	0.270 (0.179, 0.360) p < 0.001	0.006	May 1997 to January 2014 (17 years)
	DCM	570	0.001 (−0.0001, 0.002) p = NS	0.218 (0.162, 0.275) p < 0.001	0.005	April 1994 to January 2014 (20 years)

UC: ultracentrifugation. DCM: designated comparison method.

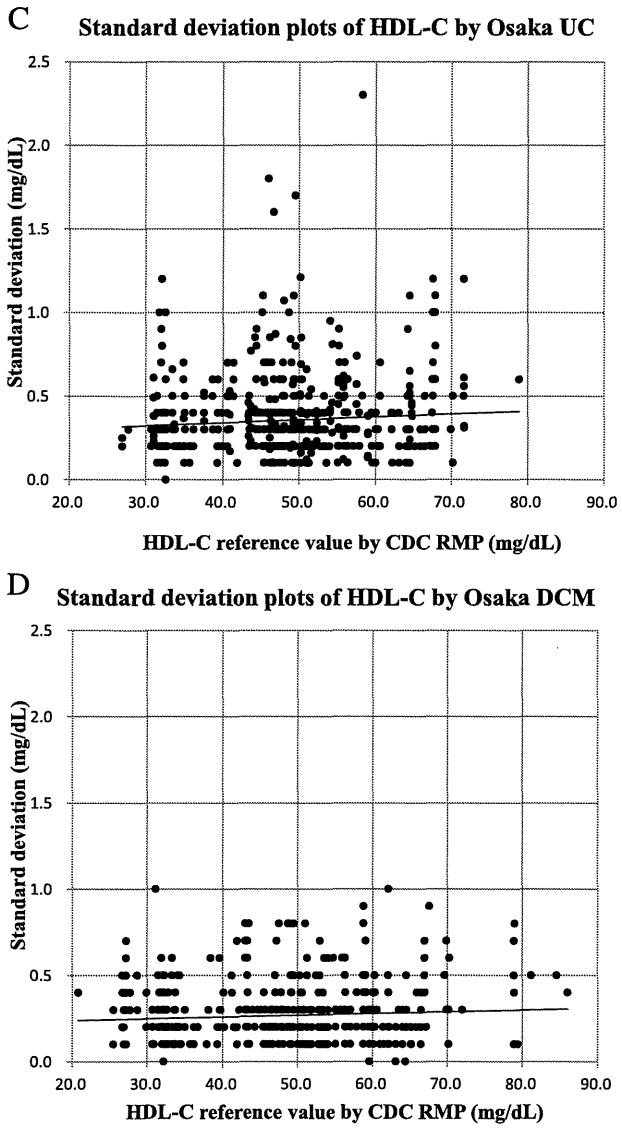

Fig. 2. C: Standard deviation plots of HDL-C by Osaka UC. The y-axis indicates the SD (mg/dl) of the Osaka UC method compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. D: Standard deviation plots of HDL-C by Osaka DCM. The y-axis indicates the SD (mg/dl) of the Osaka DCM compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. DCM: Designated comparison method.

The p-value and 95% CI for the slope were 0.083 and (−0.0001, 0.002), respectively. The p-value and 95% CI for the intercept were <0.001 and (0.162, 0.275), respectively (Table 2). The Osaka laboratory met acceptable precision goals for 100.0% (all 570 samples) within ±1 mg/dl. The maximum SD at Osaka DCM was 1.0 mg/dl (Fig. 2D).

3.3. Long-term bias (mg/dl) plots by the UC method and DCM at Osaka

Fig. 3E shows the bias (mg/dl) plots of Osaka UC HDL-C vs. CDC RMP at each run for 17 years. The minimum value of the bias was −3.7 mg/dl while the maximum value was 3.0 mg/dl. The x-axis indicated the survey run number between May 1997 and January 2014 with 154 runs and the y-axis indicated the bias (mg/dl) of Osaka UC HDL-C vs. CDC RMP. The acceptable criteria for the accuracy of HDL-C were within ±1.0 mg/dl of the target value of CDC. Each survey run consisted of 3 to 5 CDC pools for the HDL-C analysis.

Fig. 3F shows the bias (mg/dl) plots of Osaka DCM HDL-C vs. CDC RMP at each run for 20 years. The minimum value of the bias was −2.8 mg/dl while the maximum value was 2.6 mg/dl. The x-axis indicated the survey run number between April 1994 and January 2014 with 147 runs and the y-axis indicated the bias (mg/dl) of Osaka DCM HDL-C vs. CDC RMP. The acceptable criteria for the accuracy of HDL-C were within ±1.0 mg/dl of the target value of CDC. Each survey run consisted of 3 to 4 CDC pools for the HDL-C analysis.

4. Discussion

Previous epidemiological studies and clinical trials were based on the results of large scale population studies using the UC method for HDL-C, which were, in turn, based on the heparin-$MnCl_2$ precipitation method. However, an inherent problem with this precipitation method is the inability to sediment all the centrifuged lipoproteins [18,19], which mainly affects triglyceride-rich lipoproteins included in turbid or milky diseased specimens. Therefore, the UC procedure merits the elimination of interference [20,22].

High-density lipoprotein (HDL) represents a mixture of heterogeneous macromolecules and physicochemical particles. No primary certified standards or measurement procedures are currently available for HDL-C in order to establish the metrological traceability of HDL-C measurements to SI. However, UC-based CDC RMP has been the reference method of HDL-C measurements for practical use. DCM was established to better meet needs related to faster sample turnaround and higher throughput [16]. Both methods are now used to assure the accuracy of testing performed in patient care and research. However, it is important to understand the limitations of the DCM, especially with samples containing high levels of triglycerides. Therefore, it will be necessary and important to maintain the UC-based reference method and its standardization when encountering diseased and complicated samples.

Iso et al. in the Circulatory Risk in Communities Study (CIRCS) have conducted epidemiological studies on the prevention of and reductions in cerebral strokes and heart diseases among Japanese individuals for over 50 years [2,9]. During this time, we have experienced various changes for HDL-C in assay principles from the old precipitation methods to new homogeneous methods, in instruments from manual operation to automatic analyzers, in reagents from strong acids to mild enzymes, and in calibrators from cholesterol standards in alcohol to serum-based materials. All these changes have influenced the precision and accuracy of HDL-C measurements. Therefore, it is of utmost importance to ensure reference methods providing an accuracy basis for clinical measurements remain consistent and stable over time. This is achieved by maintaining a network of reference laboratories. In the present study, we assessed the measurement performance and limits of the UC and DCM methods for HDL-C at Osaka.

The homogeneous HDL-C reagents now widely adopted have several advantages: they are fully automated on various analytical instruments, have good precision, triglycerides do not need to be measured, and non-fasting samples may potentially be used. However, Miller et al. [23] and Miida et al. [24] reported some limitations when comparing these assays against the UC-RMP. Deventer et al. found that non-HDL cholesterol showed improved accuracy for cardiovascular risk score classification over that of direct or calculated LDL cholesterol in a dyslipidemic population [25]. Non-HDL-C is calculated as [total cholesterol − HDL-C]. Therefore, accurate HDL-C will be a key factor in obtaining accurate non-HDL-C values. Since non-HDL-C was previously reported to be superior to LDL-C in predicting the risk of CVD risk [8,9], it will be recommended as a primary screening test in the future by Japanese authorities.

Recent discovery that serum/plasma HDL-C markedly and selectively increased by up to 15% over the past 20 years among Japanese individuals [26] raised concerns regarding consistencies in HDL-C measurements in Japan. According to the Japanese National Health and Nutritional Survey, the average HDL-C levels of males and females

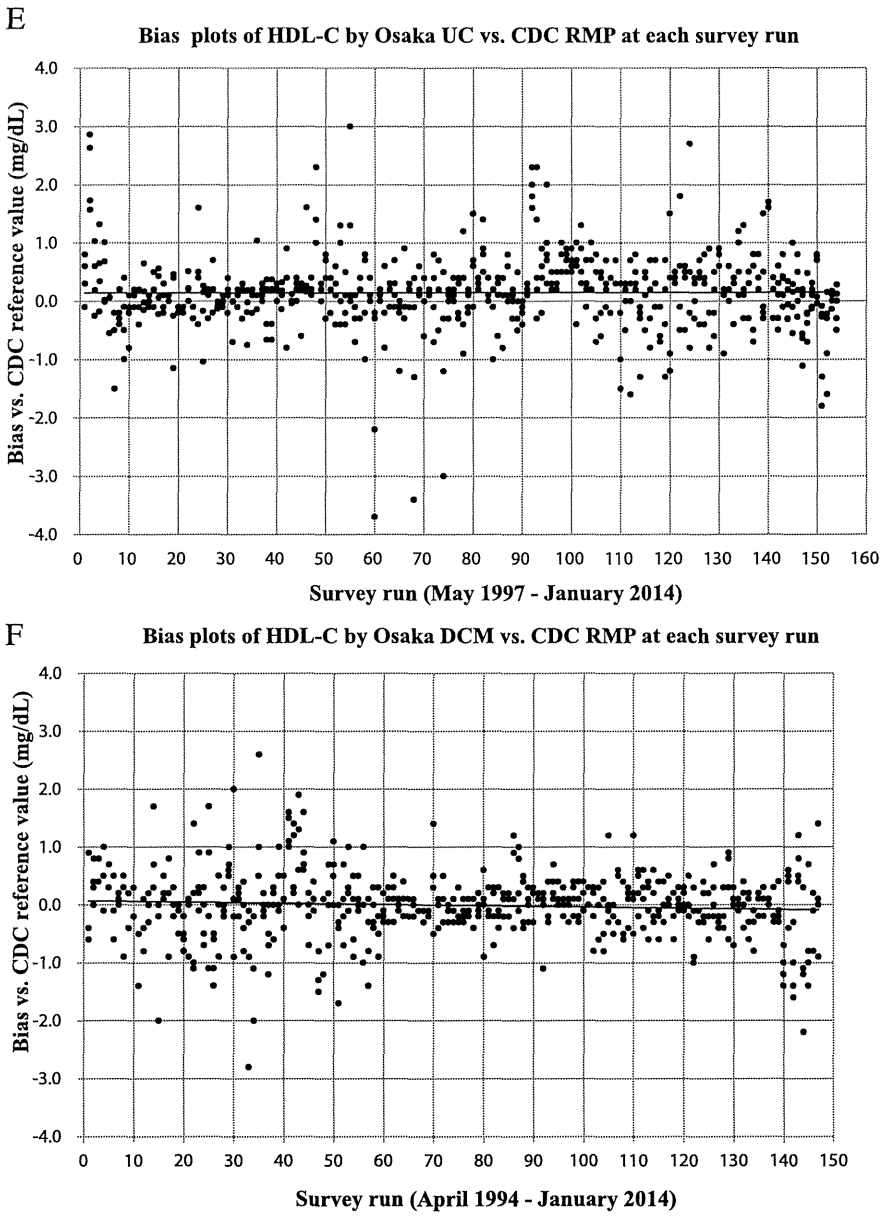

Fig. 3. E: Bias plots of HDL-C by Osaka UC vs. CDC RMP at each survey run. The y-axis indicates the bias (mg/dl) of Osaka UC compared to the CDC reference value and the x-axis indicates each survey run (May 1997–January 2014). CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. F: Bias plots of HDL-C by Osaka DCM vs. CDC RMP at each survey run. The y-axis indicates the bias (mg/dl) of Osaka DCM compared to the CDC reference value and the x-axis indicates each survey run (April 1994–January 2014). CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol.

reached 55 and 65 mg/dl in 2012, which were markedly higher than those in Western countries [27]. We tentatively concluded that this could not be attributed to a drift in the standardization of HDL-C measurements in Japan because the increase was continuous over several time points when new assay reagents and systems were introduced. Furthermore, similar findings were reported for plasma apoA-I concentrations that were independently measured [26]. However, the underlying reasons for this phenomenon and its outcome on public health in Japan remain unknown. This is a unique and perhaps important finding for world public health; therefore, it should be extensively investigated in association with recent trends and changes in various aspects of Japanese lifestyles and medical/public health environments. It is also extremely important to monitor Japanese HDL-C levels carefully for years hereafter. Therefore, methods to measure HDL-C parameters must be established based on reliable standardization and stabilization for international consistency through CRMLN activities [16].

Since 1996, 7 Japanese reagent manufacturers have developed new homogeneous methods for HDL-C to replace the old precipitation-based methods [20]. These methods present new calibration challenges

Table 3
Performance criteria applied to clinical laboratory and manufacturer for HDL-C.

Parameter	Criterion
R^2	>0.975
Bias at 40 mg/dl	≤5%
Bias at 60 mg/dl	≤5%
Average % bias	≤5%
Average absolute % bias	≤5%
Among-run CV	≤4%
t-test of bias	Not significant at $\alpha = 5\%$
Within-method outliers	1 allowed
Between-method outliers	None allowed, but may eliminate one sample

because they use different principles that include detergents or surfactants to quantify HDL-C level. Homogeneous methods that do not require a sample pretreatment step are being introduced all over Japan and are used in many clinical laboratories. Based on the HDL Cholesterol Certification Protocol for Manufacturers (November 2002) by CRMLN, we conducted protocols 10 times every 2 years since 1996 using standardized DCM as a reference to Japanese manufacturers, and all manufacturers successfully met the performance criteria (Table 3). However, further accuracy improvements in homogeneous HDL-C methods will be required in several diseased samples derived from patients with dyslipidemia [28], which may require an increased use of the UC-RMP instead of the DCM. Unsolved issues associated with homogeneous methods remain and have yet to be examined in detail.

In conclusion, the UC method and the DCM for HDL-C at Osaka were found to be highly accurate, precise, and stable for many years, and assist reagent manufacturers in calibrating their products so that clinical and epidemiological laboratories can accurately measure HDL-C for patients and in research, calculate non-HDL-C, and estimate LDL-C with the Friedewald equation. DCM is a simpler equivalent reference method that has been consistent with CDC RMP for 20 years. However, the traceability of HDL-C should be accomplished by performing a method comparison with a fresh split sample because matrix interactions can severely affect HDL-C measurements.

Acknowledgments

This work was supported by a Health and Labour Sciences Research Grant, Japan (Comprehensive Research on Lyfestyle-Related Diseases Including Cardiovascular Diseases and Daibetes Mellitus) from the Minustry of Health, Labour, and Welfare of Japan. The authors would like to thank Dr. Katsuyuki Nakajima and Dr. Ikunosuke Sakurabayashi for their valuable comments and discussion, and Ms. Yukari Ichikawa for her excellent help in providing the references and manuscript.

References

[1] Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8–15.
[2] Kitamura A, Iso H, Naito Y, et al. High-density lipoprotein cholesterol and premature coronary heart disease in urban Japanese men. Circulation 1994;89:2533–9.
[3] Okamura T, Hayakawa T, Kadowaki T, et al. The inverse relationship between serum high-density lipoprotein cholesterol level and all-cause mortality in a 9.6-year follow-up study in the Japanese general population. Atherosclerosis 2006;184:143–50.
[4] Warnick GR, 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 1995;41:1427–33.
[5] Stone NJ, Robinson J, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. J Am Coll Cardiol 2013;62:1–84.
[6] Perk J, Backer GD, Gohlke H, et al. European guidelines on cardiovascular disease prevention in clinical practice (version 2012). Eur Heart J 2012;33:1635–701.
[7] Japan Atherosclerosis Society. Japan Atherosclerosis Society (JAS) guidelines for prevention of atherosclerotic cardiovascular diseases; 2012.
[8] Okamura T, Kokubo Y, Watanabe M, et al. Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: the Suita study. Atherosclerosis 2009;203:587–92.
[9] Kitamura A, Noda H, Nakamura M, et al. Association between non-high-density lipoprotein cholesterol levels and the incidence of coronary heart disease among Japanese: the Circulatory Risk in Communities Study (CIRCS). J Atheroscler Thromb 2011;18:454–63.
[10] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[11] High-Density Lipoprotein Cholesterol Reference Method. Clinical Chemistry Standardization Activity, Division of Environmental Health Laboratory Sciences National Center for Environmental Health and Injury Control, Centers for Disease Control, Atlanta, Georgia July 1991:30333 revised July 1991.
[12] Hainline Jr A, Karon J, Lippel K, editors. Manual of laboratory operations: lipid and lipoprotein analysis., HEW Pub. No. (NIH) 75-628 (rev.), US Government Printing Office Publication No. 1982-361-132:678, 2nd ed.Bethesda, MD: National Heart, Lung and Blood Institute, Lipid Research Clinics Program; 1982.
[13] Abell LL, Levy BB, Brodie BB, et al. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952;195:357–66.
[14] Warnick GR, Albers JJ. A comprehensive evaluation of the heparin–manganese precipitation procedure for estimating high density lipoprotein cholesterol. J Lipid Res 1978;19:65–76.
[15] High density lipoprotein cholesterol designated comparison method using a modified dextran sulfate-magnesium chloride reagent, as performed by the Cholesterol Reference Method Laboratory Network, CDC; 1994.
[16] Kimberly MM, Leary ET, Cole TG, et al. Selection, validation, standardization, and performance of a designated comparison method for HDL-cholesterol for use in the Cholesterol Reference Method Laboratory Network. Clin Chem 1999;45:1803–12.
[17] Cobbaert C, Zwang L, Ceriotti F, et al. Reference standardization and triglyceride interference of a new homogeneous HDL-cholesterol assay compared with a former chemical precipitation assay. Clin Chem 1998;44:779–89.
[18] Burstein M, Scholnick HR. Lipoprotein–polyanion–metal interactions. Adv Lipid Res 1973;11:67–108.
[19] Burstein M, Legmann P. Lipoprotein precipitation. Monogr Atheroscler 1982;11:1–131.
[20] Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem 2001;47:1579–96.
[21] Method comparison and bias estimation using patient samples; approved guideline. NCCLS EP9-A, Vol. 15 No. 17; December 1995.
[22] Warnick GR, Kimberly MM, Waymack PP, et al. Standardization of measurements for cholesterol, triglycerides, and major lipoproteins. Labmedicine 2008;39:481–90.
[23] Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 2010;56:977–86.
[24] Miida T, Nishimura K, Okamura T, et al. Validation of homogeneous assays for HDL-cholesterol using fresh samples from healthy and diseased subjects. Atherosclerosis 2014;233:253–9.
[25] van Deventer HE, Miller WG, Myers GL, et al. Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. Clin Chem 2011;57:490–501.
[26] Yokoyama S, Ueshima H, Miida T, et al. High-density lipoprotein levels have markedly increased over the past twenty years in Japan. J Atheroscler Thromb 2014;21:151–60.
[27] Carroll MD, Kit BK, Lacher DA, et al. Trends in lipids and lipoproteins in US adults, 1988–2010. JAMA 2012;308:1545–54.
[28] Nakamura M, Koyama I, Iso H, et al. Measurement performance of reagent manufacturers by Centers for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network lipid standardization specified for Metabolic Syndrome-Focused Health Checkups Program in Japan. J Atheroscler Thromb 2009;16:756–63.