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Impact of the Integrated Guidance on the Care of Familial Hypercholesterolaemia

Watts G.F. *et al.* recently reported the Integrated Guidance on the Care of Familial Hypercholesterolaemia issued by the International FH Foundation[1]. On behalf of the Asia-Pacific Society of Atherosclerosis and Vascular Biology (APSAVD), we herein describe our perspective regarding the care of FH. In this summary, the authors described the guidelines for the detection, diagnosis, assessment and management of familial hypercholesterolemia (FH) in adults and children, which were determined following the discussions held at seminars and workshops at the 16th International Symposium on Atherosclerosis in Sydney in 2012. The recommended treatment is based on risk stratification, the management of non-cholesterol risk factors and the administration of safe and effective treatment to lower the LDL-cholesterol level. In addition to treatment with lipid-lowering medications, such as statins, ezetimibe, resin, fibrates and probucol, the authors described emerging therapies for FH, including mipomersen, lomitapide and anti-PCSK9 antibodies. These guidelines should have a significant impact on the management of FH in the Asia-Pacific region, as awareness of the clinical importance of FH remains very low in many countries, despite the fact that more than half of the world's population lives in this region. In the Asia-Pacific region, Japan and Australia are the only countries with published guidelines in English for the diagnosis and management of FH[2, 3], and only a few countries have published such guidelines in their mother language. FH is an autosomal dominant disease caused by the presence of abnormal LDL receptors or LDL receptor-related genes that is characterized by the triad of hyper-LDL-cholesterolemia, premature coronary artery disease (CAD) and tendon/cutaneous xanthoma. In our Japanese guidelines, we revised our diagnostic criteria for heterozygous FH, as indicated in **Table 1**, in a somewhat similar fashion to Simon Broome's criteria, although we determined the cutoff value for the LDL-cholesterol level based on the results of our multicenter study[4]. Considering that FH by itself is a very high-risk condition for CAD and that untreated patients are likely to develop CAD, such as myocardial infarction and angina pectoris, at a young age[3], providing an early diagnosis and appropriate treatment is mandatory for preventing premature death. Additionally, heterozygous FH is detected in one in 137 to 500 individuals and is one of the most frequently encountered genetic diseases in general practice[5]. Therefore, according to these guidelines, it is important to encourage the training of specialists of FH in each country and educate general practitioners regarding the diagnosis and treatment of FH. We hope that these guidelines will help to spread knowledge regarding the clinical implications of FH throughout the Asia-Pacific region and identify gaps in care, including collaborative efforts to enhance detection (especially in children), the introduction of effective early treatment, the development of country-specific models of care and the establishment of family support groups, relevant research agendas and funding mechanisms by the government and other organizations.

Table 1. Diagnostic Criteria for Heterozygous FH in Adults (Aged 15 Years or Older)[2]

1. Hyper-LDL-cholesterolemia (untreated LDL-C of ≥ 180 mg/dL)
2. Tendon xanthoma (tendon xanthoma on the backs of hands, elbows, knees, etc. or Achilles tendon hypertrophy) or xanthoma tuberosum
3. Family history of FH or premature CAD (within the second-degree relatives)

- Diagnosis should be made after excluding secondary hyperlipidemia
- If a patient meets two or three of the above-mentioned criteria, the condition should be diagnosed as FH. In the case of suspected FH, diagnosis by genetic testing is desirable.
- Xanthoma palpebrarum is not included in xanthoma tuberosum.
- Achilles tendon hypertrophy is diagnosed if the Achilles tendon thickness is ≥ 9 mm on soft X-ray imaging.
- An LDL-C of ≥ 250 mg/dL strongly suggests FH.
- If a patient is already receiving drug therapy, the lipid level that led to treatment should be used as the reference for diagnosis.
- Premature CAD is defined as CAD in men aged < 55 years or women aged < 65 years.
- If FH is diagnosed, it is preferable to also examine the patient's family members.

Conflicts of Interest

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Original Article

Revised System to Evaluate Measurement of Blood Chemistry Data From the Japanese National Health and Nutrition Survey and Prefectural Health and Nutrition Surveys

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ABSTRACT

Background: We developed a monitoring system that uses total errors (TEs) to evaluate measurement of blood chemistry data from the National Health and Nutrition Survey (NHNS) and Prefectural Health and Nutrition Surveys (PHNS).

Methods: Blood chemistry data from the NHNS and PHNS were analyzed by SRL, Inc., a commercial laboratory in Tokyo, Japan. Using accuracy and precision from external and internal quality controls, TEs were calculated for 14 blood chemistry items during the period 1999–2010. The acceptable range was defined as less than the upper 80% confidence limit for the median, the unacceptable range as more than twice the cut-off value of the acceptable range, and the borderline range as the interval between the acceptable and unacceptable ranges.

Results: The TE upper limit for the acceptable and borderline ranges was 5.7% for total cholesterol (mg/dL), 9.9% for high-density lipoprotein cholesterol (mg/dL), 10.0% for low-density lipoprotein cholesterol (mg/dL), 10.4% for triglycerides (mg/dL), 6.6% for total protein (g/dL), 7.6% for albumin (g/dL), 10.8% for creatinine (mg/dL), 6.5% for glucose (mg/dL), 9.7% for γ-glutamyl transpeptidase (U/L), 7.7% for uric acid (mg/dL), 8.7% for urea nitrogen (mg/dL), 9.2% for aspartate aminotransferase (U/L), 9.5% for alanine aminotransferase (U/L), and 6.5% for hemoglobin A1c (%).

Conclusions: This monitoring system was established to assist health professionals in evaluating the continuity and comparability of NHNS and PHNS blood chemistry data among survey years and areas and to prevent biased or incorrect conclusions.

Key words: monitoring system; accuracy; precision; total error

INTRODUCTION

In November every year, the Japanese Ministry of Health, Labour, and Welfare conducts the National Health and Nutrition Survey (NHNS) in 300 unit areas. In addition, some local governments conduct an independent Prefectural Health and Nutrition Survey (PHNS) of extended samples, according to the procedures used for the NHNS. All blood samples collected in the NHNS, and some blood samples obtained in the PHNS, are analyzed by SRL Inc., a commercial laboratory in Tokyo, Japan, and measurements are performed using the same analytic system.

All measurement is subject to error. Errors are not always constant and can differ by survey year depending on variations in many factors, including the principles underlying the method, analytic instruments, reagents, calibrator, medical technologist, and other laboratory conditions.[1,2] Even if the external and internal quality controls used at SRL are sound, measurement errors are inevitable.

The monitoring system described in this study outlines principles that can be used by physicians and other health professionals who are interested in the continuity and comparability among survey years, or in the statistical results for components of physical examinations, in the

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annual NHNS and PHNS reports. Using these principles, they can determine by themselves if the results after 2011 can be used, should be used with care, or cannot be recommended for use according to the newly established TE criteria, which are based on external and internal quality controls at SRL during the 12-year period 1999–2010. The criteria for TEs were developed for use in monitoring during 2011–2015 but not for evaluating past data. Because the results of the analysis of collected data are open to the public but information on analytic errors is not, we hoped to prevent researchers from reaching biased or incorrect conclusions in their evaluations.

In 2008, we reported tentative monitoring principles that could be used to compare blood chemistry data obtained by the NHNS.[3] However, after 2008, more PHNS data became available, to allow for evaluation of local plans in Health Japan 21. In addition, the number of blood chemistry items in the NHNS varies and has tended to increase. Finally, the Metabolic Syndrome-Focused Health Checkups Program[4] in Japan began throughout the country in 2008. Due to these developments, we decided to revise the 2008 monitoring system.

METHODS

Blood chemistry items

In this study, 14 blood chemistry items (method, unit of measure at SRL) were evaluated: total cholesterol (TC) (enzymatic, mg/dL), high-density lipoprotein cholesterol (HDL-C) (homogeneous, mg/dL), low-density lipoprotein cholesterol (LDL-C) (homogeneous, mg/dL), triglycerides (enzymatic, mg/dL), total protein (Biuret, g/dL), albumin (bromcresol green, g/dL), creatinine (enzymatic, mg/dL), glucose (enzymatic, mg/dL), γ-glutamyl transpeptidase (γ-GT, γ-GTP) (Japanese Committee for Clinical Laboratory Standards [JSCC] recommended method, U/L), uric acid (enzymatic, mg/dL), urea nitrogen (enzymatic, mg/dL), aspartate aminotransferase (AST, GOT) (JSCC recommended, U/L), alanine aminotransferase (ALT, GPT) (JSCC recommended, U/L), and hemoglobin A1c (HbA1c) (latex agglutination-turbidimetric immunoassay [LA], %).

External and internal quality control

SRL participates in the External Quality Assessment of Clinical Laboratories (EQACL) program of the Japan Medical Association (JMA)[5] and the Lipid Standardization Program of the US Centers for Disease Control and Prevention/ Cholesterol Reference Method Laboratory Network (CDC/ CRMLN). SRL also has an internal quality control system that uses 2 concentrations of quality-control materials.

Accuracy

Regarding accuracy (%bias) in Table 2, the evaluation method described in the 2010 annual report on EQACL by the JMA[5] was as follows: (1) values that deviate by 3 SDs or more from the center are removed, the mean and SD are obtained according to the measurement method used by the laboratories that participated in the survey, and the coefficient of variation (CV) is calculated according to the measurement method; (2) measurement methods are arranged in order of increasing CV; (3) measurement methods with a high rank in at least 80% of laboratories are selected; (4) the mean of data from laboratories using the measurement methods selected in the previous step is calculated, 1-way analysis of variance is used to calculate intra-method variation (expressed as SD), and a common CV is obtained; and (5) the common CV is corrected for the report unit width and a corrected common CV is obtained. Using both the adjusted mean obtained from this iterative truncation method and measurement values obtained by SRL, %bias according to samples was calculated and the mean of multiple %bias (accuracy) was calculated as an index of systematic error.[6]

Precision

Regarding precision (CV%) in Table 2, SD described in the EQACL represents dispersion in all participants, not the precision of measurement by SRL. Therefore, we were given data on the assayed values for 2 concentrations of internal quality control sera that were collected during a 1-month period, including values in November every year, randomly sampled 1 measurement value/day ($n = 1$) for 20 days, after which we calculated CV from the mean value and SD as an index of random error.[7]

Total error and relevant criteria

Subsequently, TE was calculated from accuracy and precision. Regarding total error (%) in Table 2, the equation used was "accuracy (absolute value of %bias) + precision ($1.96 \times$ CV)", which is used by the US National Cholesterol Education Program (NCEP) and the Lipid Standardization Program by CDC/CRMLN.[6] The acceptable range of TE for each blood chemistry item was defined as less than the upper 80% confidence limit for the median of the 12-year period, as calculated by the nonparametric Bootstrap method (BC_a method).[8–10] Bootstrap method analyses were conducted using SAS, version 13 (SAS Institute, Inc., Cary, NC, USA). The unacceptable range was defined as more than twice the cut-off value of the acceptable range, based on evaluation criteria adopted by the US College of American Pathologists (CAP).[11] The interval between the acceptable and unacceptable ranges was classified as the borderline range. Thus, using these TE criteria, we have created a 3-level assessment of test performance.

Use in evaluating performance in 2011

We collected the results of EQACL evaluations and SRL internal quality control data in 2011 and attempted to evaluate SRL test performance in 2011 using the proposed TE criteria.

Table 1. Annual changes in numbers of assayed samples and blood chemistry items in the National Health and Nutrition Survey in Japan

Analyte	Year												*Application in 2011*
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	
No. of assayed samples	5492	5743	5592	5413	5327	3921	3877	4319	4020	4517	4300	3930	*3515*
Total cholesterol	o	o	o	o	o	o	o	o	o	o	o	o	o
HDL cholesterol	o	o	o	o	o	o	o	o	o	o	o	o	o
LDL cholesterol	—	—	—	—	—	—	—	—	o	o	o	o	o
Triglycerides	o	o	o	o	o	o	o	o	o	o	o	o	o
Total protein	o	o	o	o	o	o	o	o	o	o	o	o	o
Albumin	—	—	—	—	o	o	o	o	o	o	o	o	o
Creatinine	—	o	—	—	—	—	—	—	—	o	o	o	o
Glucose	o	o	o	o	o	o	o	o	o	o	o	o	o
γ-GT (γ-GTP)	—	o	—	—	—	—	—	—	—	—	—	o	o
Uric acid	—	o	—	—	—	—	—	—	—	—	—	o	o
Urea nitrogen	—	o	—	—	—	—	—	—	—	—	—	—	—
AST (GOT)	—	—	—	—	—	—	—	—	—	—	—	o	o
ALT (GPT)	—	—	—	—	—	—	—	—	—	—	—	o	o
HbA1c	—	—	—	o	o	o	o	o	o	o	o	o	o

White circles show blood chemistry items assayed in the corresponding year.
Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; γ-GT (γ-GTP), γ-glutamyl transpeptidase; AST (GOT), aspartate aminotransferase; ALT (GPT), alanine aminotransferase; HbA1c, hemoglobin A1c.

Criteria for CDC/CRMLN lipid standardization

To evaluate lipid measurement, the following NCEP criteria were used: TC—accuracy within 3% of target value for CDC/CRMLN reference measurement procedure, precision as CV of 3% or less, and TE of 9% or less; HDL-C—accuracy within 5% of target value, precision as CV 4% or less, and TE of 13% or less; LDL-C—accuracy within 4% of target value, precision as CV of 4% or less, and TE of 12% or less.[12]

Implementation survey for PHNS

In 2007, our study group surveyed prefectural governments regarding implementation of their PHNS, including dietary intake surveys and blood examination, and collected additional data on the number of blood samples they entrusted to SRL for analysis in 2011.[13]

RESULTS

Table 1 shows annual changes in blood chemistry items measured and number of analyzed NHNS samples assayed at SRL during 1999–2010. Items measured every year since 1999 were TC, HDL-C, triglycerides, total protein, and glucose. LDL-C, albumin, creatinine, and HbA1c were recently added to these 5 items. Other items, such as γ-GT (γ-GTP), uric acid, urea nitrogen, AST (GOT), and ALT (GPT), have been measured infrequently. The average number of assayed samples in the NHNS was 4704 during 1999–2010.

Table 2 shows measurement performance at SRL, based on the EQACL of the JMA. On the basis of these calculations, criteria for acceptable, borderline, and unacceptable ranges were established, as shown in the column labeled Proposed TE Criteria.[10] The upper limit of TE in the new acceptable and borderline ranges for each item was 5.7% for TC, 9.9% for HDL-C, 10.0% for LDL-C, 10.4% for triglycerides, 6.6% for total protein, 7.6% for albumin, 10.8% for creatinine, 6.5% for glucose, 9.7% for γ-GT (γ-GTP), 7.7% for uric acid, 8.7% for urea nitrogen, 9.2% for AST (GOT), 9.5% for ALT (GPT), and 6.5% for HbA_{1C}. Concerning the acceptable TE range, 50% of the evaluation limits (1 side) of the CAP evaluation criteria, which are widely used worldwide, was adopted and is shown as a reference in the column labeled CAP TE in Table 2.[11] TE criteria for HbA_1c were not established in the CAP survey. Although the acceptable range for γ-GT (γ-GTP) is expressed as SD in the CAP evaluation criteria, 7.5% was used as the corresponding value.

A 2007 implementation survey showed that 25 (53.2%) of the 47 prefectures in Japan independently performed blood examinations. Blood examinations were entrusted to SRL by 21 of the 25 prefectures and to a local laboratory by the other 4. A total of 15 096 samples from the 21 prefectures were analyzed by SRL. This number was 3.2 times the mean sample number (4704) of the NHNS (Table 1). Additionally, according to the 2011 survey, 20 (42.6%) of the 47 prefectures performed blood examinations.

Blood examinations were entrusted to SRL by 15 of the 20 prefectures and to a local laboratory by the other 5. A total of 7063 samples from the 15 prefectures were analyzed by SRL. This number was 1.5 times the average sample number of the NHNS (Table 1). The survey of the current situation in each prefecture was not conducted systematically, and measurement items are different for each prefecture.

In 2011, urea nitrogen was not assayed in the NHNS or PHNS; thus, there was a total of 13 items. When TE was calculated for each SRL item in 2011 to establish proposed TE

Table 2. SRL performance based on JMA external quality assessment and SRL internal quality control system (unit, %)

Analyte	Performance	Measurement performance by SRL during observation period: Year 1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Median (LL, UL of 80% CL)	Proposed TE Criteria: Acceptable	Borderline	Unacceptable	*Application to new data: Performance in 2011*	*Evaluation by proposed TE criteria in 2011*	(For reference) CAP TE Criteria
Total cholesterol	Accuracy (%bias)	0.19	−0.48	0.27	0.34	−0.15	−0.06	0.13	−0.82	−1.31	−1.45	−0.82	−0.66	−0.32 (−0.74, 0.04)				*0.19*		
	Precision (CV%)	1.7	1.6	1.3	1.1	1.6	1.0	1.2	1.0	0.7	0.8	0.7	0.7	1.1 (0.9, 1.3)				*0.8*		
	Total Error (%)	3.6	3.6	2.7	2.5	3.3	2.1	2.4	2.7	2.7	3.0	2.2	2.0	2.7 (2.5, 2.9)	<2.9	2.9–5.7	≥5.8	*1.8*	*acceptable*	5.0
HDL cholesterol	Accuracy (%bias)	−0.19	−1.57	−1.09	1.60	0.02	−0.33	0.70	1.29	−2.89	−0.90	−0.17	−0.68	−0.26 (−0.79, −0.08)				*−2.00*		
	Precision (CV%)	2.4	1.8	1.6	2.1	2.0	1.5	1.6	2.3	1.5	1.8	1.3	1.7	1.8 (1.6, 1.9)				*1.7*		
	Total Error (%)	4.9	5.1	4.2	5.7	4.0	3.2	3.8	5.7	5.8	4.4	2.7	4.0	4.3 (4.0, 5.0)	<5.0	5.0–9.9	≥10.0	*5.3*	*Borderline*	15.0
LDL cholesterol	Accuracy (%bias)	—	—	—	—	—	—	—	—	−0.39	1.95	−2.45	0.50	0.06 (−1.42, 1.23)				*0.63*		
	Precision (CV%)	—	—	—	—	—	—	—	—	1.2	2.0	0.9	1.4	1.3 (1.1, 1.7)				*1.1*		
	Total Error (%)	—	—	—	—	—	—	—	—	2.7	5.9	4.2	3.2	3.7 (3.0, 5.0)	<5.0	5.0–10.0	≥10.1	*2.8*	*acceptable*	15.0
Triglycerides	Accuracy (%bias)	1.91	−0.58	−1.34	0.37	1.56	−0.12	−0.36	0.00	−0.97	−1.10	−1.86	−1.67	−0.47 (−1.04, −0.06)				*−0.18*		
	Precision (CV%)	1.8	2.3	2.4	2.6	2.3	1.5	1.4	2.3	1.0	1.0	1.1	1.2	1.7 (1.3, 2.3)				*1.6*		
	Total Error (%)	5.5	5.2	6.1	5.5	6.2	3.0	3.1	4.6	2.9	3.1	4.0	4.0	4.3 (3.6, 5.3)	<5.3	5.3–10.4	≥10.5	*4.4*	*acceptable*	12.5
Total protein	Accuracy (%bias)	−0.27	−0.12	0.46	−0.24	−0.14	−0.28	0.19	−0.07	−0.39	1.59	−0.58	1.78	−0.13 (−0.26, 0.06)				*3.21*		
	Precision (CV%)	1.4	1.0	0.9	1.5	2.0	1.6	1.4	1.5	1.5	1.6	1.0	1.3	1.5 (1.4, 1.5)				*1.3*		
	Total Error (%)	3.0	2.1	2.2	3.2	4.1	3.4	2.9	3.0	3.3	4.7	2.5	4.3	3.1 (3.0, 3.4)	<3.4	3.4–6.6	≥6.7	*5.8*	*Borderline*	5.0
Albumin	Accuracy (%bias)	−2.43	−0.75	0.45	−1.12	0.64	0.12	−0.06	0.11	1.05	−0.28	−1.14	0.46	0.03 (−0.52, 0.29)				*5.19*		
	Precision (CV%)	1.7	1.3	2.0	1.8	1.9	1.2	1.6	1.1	0.9	1.2	1.0	1.2	1.3 (1.2, 1.6)				*1.0*		
	Total Error (%)	5.8	3.3	4.4	4.6	4.4	2.5	3.2	2.3	2.8	2.6	3.1	2.8	3.1 (2.8, 3.8)	<3.8	3.8–7.6	≥7.7	*7.1*	*Borderline*	5.0
Creatinine	Accuracy (%bias)	−2.24	1.93	−0.08	−0.34	0.15	0.19	−0.76	−0.55	−0.76	−1.25	−0.54	−4.18	−0.55 (−0.76, −0.21)				*−2.77*		
	Precision (CV%)	1.5	2.6	3.7	2.0	1.9	2.3	1.8	2.3	1.7	2.3	1.3	1.8	2.0 (1.8, 2.3)				*1.7*		
	Total Error (%)	5.1	7.1	7.2	4.3	3.9	4.8	4.3	5.0	4.1	5.8	3.1	7.7	4.9 (4.3, 5.5)	<5.5	5.5–10.8	≥10.9	*6.1*	*Borderline*	7.5
Glucose	Accuracy (%bias)	0.42	−0.58	−0.39	−0.31	0.17	−0.06	0.76	0.53	−0.83	−0.04	0.01	−0.74	−0.05 (−0.35, 0.09)				*−0.47*		
	Precision (CV%)	1.4	1.0	1.7	1.2	1.4	1.4	1.4	1.5	1.5	0.8	0.8	1.0	1.4 (0.8, 0.8)				*1.1*		
	Total Error (%)	3.1	2.5	3.7	2.7	3.0	2.7	3.5	3.5	3.8	1.6	1.6	2.7	2.9 (2.7, 3.3)	<3.3	3.3–6.5	≥6.6	*2.6*	*acceptable*	5.0
γ-GT (γ-GTP)	Accuracy (%bias)	0.74	−0.01	−0.24	0.82	0.37	−0.13	−0.48	−0.83	−1.50	0.45	−0.75	−1.04	−0.19 (−0.62, 0.18)				*−1.39*		
	Precision (CV%)	1.8	1.8	1.6	1.7	2.3	1.3	2.0	2.1	1.9	2.0	2.5	2.1	2.0 (1.8, 2.1)				*1.8*		
	Total Error (%)	4.2	3.5	3.4	4.2	4.8	2.7	4.4	5.0	5.2	4.4	5.7	5.2	4.4 (4.2, 4.9)	<4.9	4.9–9.7	≥9.8	*4.9*	*acceptable*	7.5
Uric acid	Accuracy (%bias)	0.21	−0.59	−0.43	0.25	−0.26	0.81	−0.44	0.88	−0.44	−0.56	0.31	1.26	−0.03 (−0.44, 0.28)				*1.11*		
	Precision (CV%)	2.1	2.1	1.4	1.5	1.4	1.4	1.8	1.5	1.6	1.1	1.3	1.6	1.5 (1.1, 1.1)				*1.1*		
	Total Error (%)	4.4	4.8	3.2	3.2	3.1	3.6	4.0	3.8	3.6	2.7	2.9	4.4	3.6 (3.2, 3.9)	<3.9	3.9–7.7	≥7.8	*3.3*	*acceptable*	8.5
Urea nitrogen	Accuracy (%bias)	−1.69	0.16	0.25	1.74	−0.17	0.75	−0.33	0.69	−2.86	—	—	1.58	0.21 (−0.25, 0.69)				*not assayed*		
	Precision (CV%)	1.3	1.2	1.2	1.7	1.8	1.1	1.9	1.4	1.5	—	—	1.5	1.5 (1.3, 1.6)				*not assayed*		
	Total Error (%)	4.3	2.6	2.7	5.1	3.7	3.0	4.1	3.4	5.8	—	—	4.5	3.9 (3.3, 4.4)	<4.4	4.4–8.7	≥8.8	*not assayed*		4.5
AST (GOT)	Accuracy (%bias)	3.03	−0.43	0.21	−0.07	1.37	0.59	−0.60	0.25	−1.25	0.51	0.71	0.64	0.38 (0.07, 0.62)				*−0.37*		
	Precision (CV%)	1.7	1.8	1.3	1.1	2.1	1.4	1.9	1.5	2.2	1.5	1.6	2.2	1.7 (1.5, 1.9)				*1.8*		
	Total Error (%)	6.3	4.0	2.7	2.3	5.5	3.4	4.4	3.3	5.6	3.5	3.8	5.0	3.9 (3.4, 4.6)	<4.6	4.6–9.2	≥9.3	*3.9*	*acceptable*	10.0
ALT (GPT)	Accuracy (%bias)	2.81	−0.22	0.38	−1.43	−0.08	1.48	1.06	−0.64	−1.47	0.95	0.88	0.37	0.38 (−0.15, 0.92)				*−1.12*		
	Precision (CV%)	1.4	1.7	1.4	1.4	2.3	1.5	2.3	2.2	2.2	1.6	1.8	2.2	1.8 (1.6, 2.2)				*2.3*		
	Total Error (%)	5.5	3.6	3.2	4.2	4.5	4.4	5.5	4.9	5.8	4.1	4.4	4.7	4.5 (4.3, 4.8)	<4.8	4.8–9.5	≥9.6	*5.6*	*Borderline*	10.0
HbA_1c	Accuracy (%bias)	—	—	−0.39	0.52	0.01	2.25	1.01	1.28	−0.34	−1.08	−0.14	−0.26	−0.07 (−0.30, 0.52)				*0.12*		
	Precision (CV%)	—	—	1.1	1.1	1.0	1.2	1.1	1.0	1.4	1.2	1.4	1.6	1.2 (1.1, 1.3)				*2.0*		
	Total Error (%)	—	—	2.5	2.7	2.0	4.6	3.2	3.2	3.1	3.4	2.9	3.4	3.1 (2.8, 3.3)	<3.3	3.3–6.5	≥6.6	*4.0*	*Borderline*	

Accuracy as an index of systematic error is expressed as %bias calculated based on JMA criteria.
Precision as an index of random error is expressed as CV calculated from SRL internal quality control data.
Total error is calculated as the sum of accuracy and precision, ie, absolute value of %bias + 1.96 × CV.
Abbreviations: JMA, Japan Medical Association; CAP, College of American Pathologists; TE, total error; LL, lower limit; UL, upper limit; CL, confidence limit; HDL, high-density lipoprotein; LDL, low-density lipoprotein; γ-GT (γ-GTP), γ-glutamyl transpeptidase; AST (GOT), aspartate aminotransferase; ALT (GPT), alanine aminotransferase; HbA1c, hemoglobin A1c.

Table 3. SRL performance based on CDC/CRMLN Lipid Standardization Program (unit, %)

Analyte	Performance	CDC Criteria	Year											Average	SD
			1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010		
Total cholesterol	Accuracy (%bias)	±3.0	0.00	−1.30	0.00	−0.90	0.30	−0.10	−0.90	−0.90	−0.90	−0.30	−0.50	0.10	−0.45	0.52
	Precision (CV%)	3.0	0.5	0.6	0.6	0.5	0.5	0.6	0.4	0.4	0.4	0.5	0.4	0.3	0.48	0.10
	Total Error (%)	9.0	1.0	2.5	1.2	1.9	1.3	1.4	1.7	1.7	1.7	1.3	1.3	0.8	1.48	0.45
HDL cholesterol	Accuracy (%bias)	±5.0	0.70	0.70	2.00	2.00	1.00	1.00	1.20	1.20	1.20	−1.00	0.00	0.00	0.83	0.85
	Precision (CV%)	4.0	1.0	1.0	1.3	1.3	1.7	1.7	1.1	1.1	1.1	1.0	0.7	0.7	1.14	0.32
	Total Error (%)	13.0	2.7	2.7	4.6	4.6	4.4	4.4	3.4	3.4	3.4	3.0	1.4	1.4	3.28	1.12
LDL cholesterol	Accuracy (%bias)	±4.0				−0.60	−0.60	−0.70	−0.70	0.30	0.30	1.70	−1.40	−1.40	−0.34	0.98
	Precision (CV%)	4.0				1.2	1.2	0.7	0.7	0.4	0.4	0.6	0.6	0.6	0.71	0.30
	Total Error (%)	12.0				3.0	3.0	2.1	2.1	1.1	1.1	2.9	2.6	2.6	2.28	0.75

Accuracy as an index of systematic error is expressed as %bias calculated based on CDC criteria.
Precision as an index of random error is expressed as CV calculated based on lipid standardization criteria of CDC.
Total error is calculated as the sum of accuracy and precision, ie, absolute value of %bias + 1.96 × CV.
Abbreviations: CDC, Centers for Disease Control and Prevention; CRMLN, Cholesterol Reference Method Laboratory Network; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

criteria, the evaluation was acceptable for 7 items (53.8%) —TC, LDL-C, triglycerides, glucose, γ-GT (γ-GTP), uric acid, and AST (GOT)—and borderline for 6 items (46.2%), namely, HDL-C, total protein, albumin, creatinine, ALT (GPT), and HbA_1c. No item was evaluated as unacceptable (Table 2).

Table 3 shows the measurement performance of SRL for TC, HDL-C, and LDL-C, based on the criteria of the Lipid Standardization Program by CDC/CRMLN. In each standardization year, performance satisfied the CDC/CRMLN criteria for clinical laboratories.

DISCUSSION

In standardization—the most advanced system of quality control assessment—target values are obtained by using globally accepted definitive or reference measurement procedures. However, in the EQACL, measurement values are collected from all participants and, after statistical analysis, adjusted mean values are obtained and used as an index of accuracy. A similar data processing method is used in external quality control assurance programs in Western countries.[14,15] This method statistically excludes extreme outliers and misreports, which improves the reliability of adjusted mean values as indices of accuracy. Such adjusted means do not represent physicochemical accuracy, as such, but are often used for practical purposes as consensus values in clinical surveys. Consensus values are often used as a substitute for accuracy when there is no established reference method, or when a reference method exists but is not used due to its complexity or technical difficulty. In this respect, we have no objection to the use of consensus values at many laboratories, such as those derived from approximately 3000 participants in the EQACL of the JMA.[5]

The sources of error in measured values include changes in: the underlying principles of the measurement method, analytic devices, sample status (fresh, frozen), reagents or reagent reactivity, calibrators and their value assignments, the skill of analytical technologists, and other laboratory conditions.[1,2,5,6] Measurement error can result in clinical examination-derived discontinuities with previously obtained results in surveys (such as retrospective case-control studies), which could markedly affect annual follow-up. In this study, we conducted detailed follow-up surveys of these factors to avoid discontinuities derived from clinical examinations. A disadvantage of using the mean value of an external quality assessment as an index of accuracy is that the method routinely used during each period has a direct influence on measurement values. For example, when an analytic method based on new measurement principles is developed and adopted at clinical laboratories, due to convenience and/or cost and time savings, changes in mean value are sometimes observed along with analytic errors.

Case 1: The routine analytic method for HDL-C changed from a precipitation method using polyanions and cations to a homogeneous method using detergent or surfactant. The new method has been adopted by many laboratories, and age-related changes in mean HDL-C values have been reported since the switch. In this former case, changes in mean HDL-C values were observed and, as a consequence, analytic errors change.[16–19]

Case 2: There has been increasing demand for more-precise creatinine analysis for people with diabetes mellitus and renal disorders, and the calibrator is changing from the old, water-soluble standard to a new serum-based reference material with high accuracy, as confirmed by gas chromatography/isotope dilution/mass spectrometry. Additionally, in many laboratories the creatinine method has changed from the classic Jaffe method to newly developed enzymatic methods. Changes in mean creatinine values have been observed with these new methods and, inevitably, analytic errors also change.[20,21]

The survey protocol agreed by the Ministry of Health, Labour, and Welfare in Japan and SRL stipulates that the same analytic system for the NHNS (BioMajesty 8060 device No. 1, JEOL Ltd.; installed in the SRL Medical Ultimate Quality Service [MUQS] Laboratory) should also be used for

blood examinations that are independently entrusted by prefectures to SRL. This protocol allows PHNS and NHNS results to be monitored in the same manner and permits PHNS data to be added to NHNS. The sample numbers of the PHNS are generally larger than those of the NHNS. However, there are 2 limitations in the use of PHNS data: the measured items differ according to prefecture, and it is possible that the analytic laboratory was changed from SRL to a local laboratory or from a local laboratory to SRL. Therefore, before using PHNS results as additional data, the laboratory responsible for the results should be confirmed. In this study, only samples measured by SRL were included.

In this study, on the basis of quality control results, target TE values for the subsequent 5 years were determined. Specifically, the acceptable limit was defined as the upper 80% confidence limit of TE. TE values above this limit were considered to be in the borderline or unacceptable range, and a caution was issued. The probability of including borderline or unacceptable ranges using these target values remains at 10% even if performance remains equal to that during the previous 12-year period. Assuming annual improvements in performance, approximately 50% of TE values in the subsequent 5-year period are expected to be within the acceptable range. In quality control, there are no absolute criteria for quality, and quality is improved by daily efforts to repeatedly establish and meet criteria. Our monitoring system uses past data to establish target values for a subsequent 5-year period, and adjustments are made by revising target values at 5-year intervals. The system is thus compatible with the idea of quality control. The TE limit for the acceptable and borderline ranges was established for monitoring during 2011–2015, not for its application to past data. Application to the year 2011 (Table 2) confirms the suitability of the proposed TE criteria. When TE falls within the acceptable or borderline ranges, annual continuity and comparability of survey results can be regarded as satisfactory. However, when TE falls within the unacceptable range, measurement values should be used with caution.

Precision is an index of the reproducibility of measurement values obtained by a laboratory. In this study, since TE was calculated using an equation, CV was limited to a singlicate value ($n = 1$) in internal quality control sera for 20 days. CV was calculated from 2 types of commercially available internal quality control serum in SRL. However, if there was a difference of 10% or more in CV between the concentrations of internal quality control materials, the higher CV was used.[7]

In lipid standardization by CDC/CRMLN,[12] the accuracy, precision, and TE for SRL measurements of TC, HDL-C, and LDL-C met CDC criteria (Table 3) for clinical laboratory use. Therefore, concerning these 3 lipid items, all results in the NHNS and the results in some PHNS can be compared with results in Western countries. However, only results obtained during the previous 9-year period are available for LDL-C, and it is desirable to use these results as a reference.

In conclusion, we used TE criteria to develop a revised 3-level assessment of test performance and evaluated the continuity and comparability of 14 blood chemistry items assayed at SRL for the NHNS and PHNS in Japan. To further improve reliability, TE performance criteria should be updated every 5 years.

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ONLINE ONLY MATERIALS

The Japanese-language abstract for articles can be accessed by clicking on the tab labeled Supplementary materials at the journal website http://dx.doi.org/10.2188/jea.JE20120032.

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LDL cholesterol performance of beta quantification reference measurement procedure ☆

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ABSTRACT

Background: Accurate measurement of blood lipids is crucial in cardiovascular disease risk management. The Centers for Disease Control and Prevention (CDC) Cholesterol Reference Method Laboratory Network (CRMLN) has assured the accuracy of these measurements for over 20 years using beta quantification (BQ) method as reference measurement procedure (RMP) for high- and low-density lipoprotein cholesterol (HDL-C, LDL-C). Only limited data exist about the performance of the BQ RMP.
Methods: Bottom fraction cholesterol (BFC), HDL-C, and LDL-C results after ultracentrifugation from the CDC lipid reference laboratory and the Japanese CRMLN laboratory were compared using 280 serum samples measured over the past 15 years. Data were compared statistically using method comparison and bias estimation analysis.
Results: Regression analysis between CDC (x) and Osaka (y) for BFC, HDL-C, and LDL-C were $y = 0.988x + 1.794$ ($R^2 = 0.997$), $y = 0.980x + 1.118$ ($R^2 = 0.994$), and $y = 0.987x + 1.200$ ($R^2 = 0.997$), respectively. The Osaka laboratory met performance goals for 90% to 95% of the CDC reference values.
Conclusions: The BQ method by the Osaka CRMLN laboratory is highly accurate and has been stable for over 15 years. Accurate measurement of BFC is critical for the determination of LDL-C.

1. Introduction

Increased concentrations of low-density lipoprotein cholesterol (LDL-C) are associated with an increased risk for the development of cardiovascular diseases (CVDs), especially coronary heart disease (CHD) [1,2]. Other major risk factors include hypertension, diabetes mellitus, smoking, and chronic kidney diseases [3,4]. Interventions to decrease LDL-C levels can improve the risk of CVD and result in reductions in atherosclerotic lesions [5–8]. Because of the strong and positive association between LDL-C and CVD, 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults [9], the Third Report of the U.S. National Cholesterol Education Program (NCEP) [10,11], the European Atherosclerosis Society [12], and Japan Atherosclerosis Society Guidelines for the Prevention of Atherosclerotic Cardiovascular Diseases 2012 [13] focused primarily on LDL-C for the categorization and treatment of dyslipidemia. Thus, measuring LDL-C has been the cornerstone of cardiovascular risk assessment and prevention for the past decades.

The precise and accurate measurement of LDL-C is of particular importance for correctly and consistently classifying individuals at risk for CVD as outlined in clinical guidelines for subsequent treatment of patients. The precision and accuracy of LDL-C measurements needed to assure that appropriate patient care was established by the NCEP [14]. The beta quantification (BQ) procedure, which relies on ultracentrifugation (UC) to separate apo B lipoprotein (apo B) particles

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Table 1
Performance criteria applied to CRMLN lipid reference laboratory using BQ RMP.

Lipid	Precision	Accuracy
BFC	CV ≤ 1.5%	±(CDC LDL-C reference value × 0.02 + HDL-C bias vs. CDC) [max = ±2 mg/dL or 0.04 (HDL-C reference value) if smaller]
HDL-C	SD ≤ 1 mg/dL	±CDC HDL-C reference value × 0.04
LDL-C	CV ≤ 1.5%	±CDC LDL-C reference value × 0.02

CRMLN: Cholesterol Reference Method Laboratory Network. BQ RMP: Beta quantification reference measurement procedure.
CDC: US Centers for Disease Control and Prevention.
BFC: Bottom fraction cholesterol.

according to the hydrated density at d = 1.006, has been the established reference measurement procedure (RMP) for HDL-C and LDL-C [15,16]. BQ RMP performed at the U.S. Centers for Disease Control and Prevention (CDC) and Cholesterol Reference Method Laboratory Network (CRMLN) is considered the highest order RMP for this analyte. For over 15 years, the National Cerebral and Cardiovascular Center at Osaka, Japan has standardized their LDL-C BQ RMP through participation in the CRMLN. Members of the CRMLN are required to meet stringent performance criteria for precision and accuracy to allow both calibration and calibration verification of routine assays. Few reports are available on the performance of BQ RMP.

Using data obtained between May 1997 and October 2012, the precision and accuracy for HDL-C and LDL-C as measured at the Osaka laboratory were determined. We determined the fixed and/or proportional bias and correlations between the CDC and Osaka laboratories, and assessed factors that may affect results obtained with the BQ method by verifying relationships among bottom fraction cholesterol (BFC) — one major component of the BQ procedure, HDL-C, and LDL-C.

2. Material and methods

2.1. Materials

All materials were prepared according to Clinical Laboratory Standards Institute (CLSI) document C37-A. This implies that no preservatives or no additives were added. In this study, 67 different pool concentrations (lots) were used among the 280 survey samples provided by the CDC as part of the CRMLN monitoring surveys. One lot (bq47) was used 8 times over 2.5 years, which represented the longest period any lot was used. All CDC survey pools were blinded to the CRMLN participants. The pools were shipped frozen and stored at −70 °C before BQ analysis, and they were analyzed between May 1997 and October 2012 in 70 survey runs, with each survey run consisting of 3 to 5 different pools.

Measurements 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 since April 2012 (all laboratories are referred to as 'Osaka laboratory').

2.2. Ultracentrifugation

BQ employs preparative ultracentrifuge (Beckman Coulter, Optima L-70K) to remove the chylomicrons and very-low-density lipoproteins (VLDL) of apo B-containing lipoproteins [17]. The methods at CDC and Osaka used 5 ml of serum per sample at a density of d = 1.006 kg/L (0.195 mol/L NaCL solution) and a 50.4 Ti rotor (Beckman Coulter) for UC. UC was carried out at CDC for 16.2 hours at 120,000 ×g, and 18 °C, and at Osaka for 18.5 hours, 105,000 ×g, and 18 °C. After UC, chylomicrons and VLDL in the top fraction (d < 1.006 kg/L) were removed and the remaining bottom fraction (d > 1.006 kg/L) including high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) (Lp(a)) was quantitatively transferred to a 5.00 mL volumetric flask and adjusted for volume with 0.15 mol/L NaCL solution [14,15]. The total cholesterol in this bottom fraction (BFC) was determined from one aliquot.

2.3. HDL-C precipitation

One mL aliquots of the apo B-containing lipoproteins in the bottom fraction were precipitated with 40 μL heparin (sodium injection, 5000 USP units/mL, Baxter Healthcare Corp.) and 50 μL manganese reagents (manganese(II) chloride solution, 1.00 mol/L ± 0.01 mol/L, SIGMA). The precipitate was removed by centrifugation for 30 min at 1500 ×g, 4 °C [18]. HDL-C was determined in the supernatant in duplicate measurements by the Abell–Kendall RMP [19]. LDL-C was calculated as the difference between BFC and HDL-C. A total of 8 replicate values per sample were obtained, and the mean of these replicates is used for comparison of assay performance.

2.4. Performance criteria

Performance criteria applied to the CRMLN lipid reference laboratories are summarized in Table 1. Because the LDL-C is the difference between BFC and HDL-C, the bias criterion for BFC was determined by the allowable bias for LDL-C and HDL-C and was considered to be ± the sum of the allowable HDL-C and LDL-C bias.

Table 2
Measurement performance of the CRMLN laboratory at Osaka determined with 280 pooled sera measured between May 1997 and October 2012 in 70 survey runs.

Statistical item	BFC	HDL-C	LDL-C
Mean precision as %CV (SD)	0.60 (0.342)	1.01 (0.605)	0.85 (0.461)
Mean bias as % (SD)	−0.12 (0.853)	0.45 (1.708)	−0.34 (1.148)
Pass rate for imprecision (N)	95.4% (267)	95.4% (267)	91.8% (257)
Pass rate for bias (N)	91.4% (256)	94.6% (256)	89.6% (251)
Absolute bias (%)	0.63 ± 0.589	1.23 ± 1.270	0.86 ± 0.830
Bias in mg/dL (95% CI)	0.34 (0.14, 0.53)	−0.16 (−0.26, −0.07)	0.49 (0.32, 0.66)
Limits of agreement in mg/dL	−2.87 to 3.54	−1.76 to 1.43	0.31 to 0.66
Slope (95% CI)	0.988 (0.981, 0.995)	0.980 (0.971, 0.989)	0.987 (0.980, 0.993)
Intercept (95% CI)	1.794 (0.581, 3.006)	1.118 (0.676, 1.560)	1.200 (0.388, 2.011)
Correlation coefficient as R^2	0.997	0.994	0.997

CRMLN: Cholesterol Reference Method Laboratory Network.
BFC: Bottom fraction cholesterol.

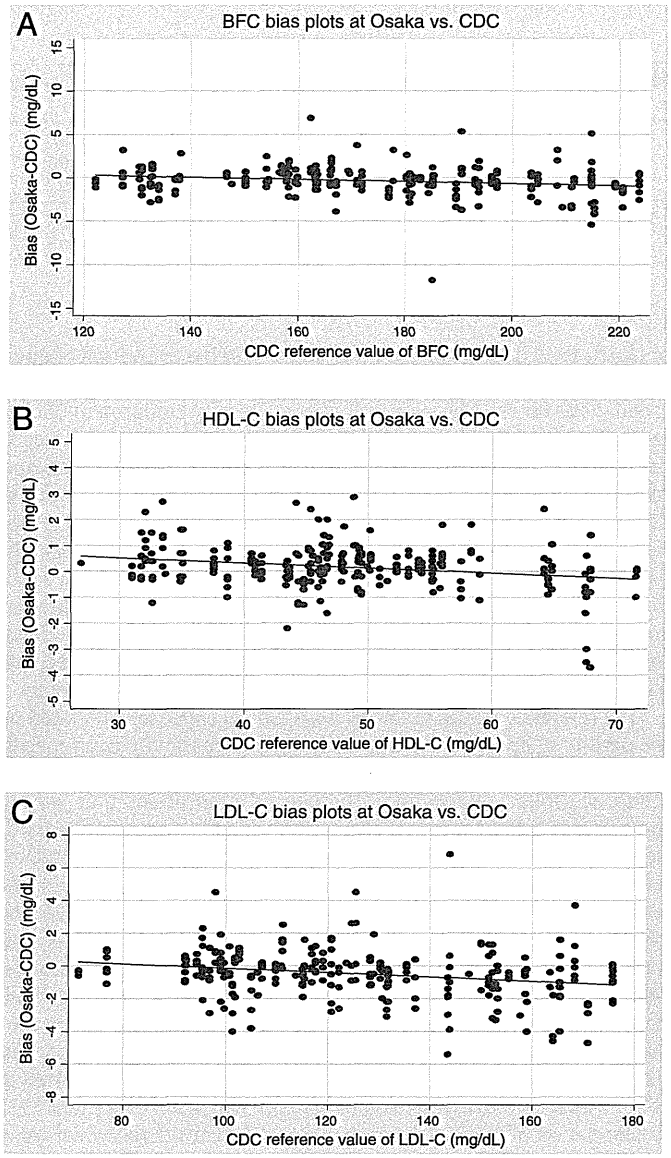

Fig. 1. Scatter plots of bias at Osaka vs. CDC for BFC (A), HDL-C (B) and LDL-C (C). (A) CDC: US Centers for Disease Control and Prevention. BFC: Bottom fraction cholesterol. x-axis indicates CDC reference value of BFC (unit: mg/dL) in the concentration range from 122.3 to 223.7 mg/dL and y-axis indicates the BFC bias between Osaka and CDC (unit: mg/dL). y (bias (Osaka–CDC)) = −0.012 × (CDC reference value) + 1.759 [n: 280, R^2 = 0.042 (p-value: 0.001)]. p-value and 95% CI are 0.001 and (−0.019, −0.005) for slope, respectively. p-value and 95% CI are 0.004 and (0.551, 2.968) for intercept, respectively. (B) CDC: US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. x-axis indicates CDC reference value of HDL-C (unit: mg/dL) in the concentration range from 27.0 to 72.4 mg/dL and y-axis indicates the HDL-C bias between Osaka and CDC (unit: mg/dL). y (bias (Osaka–CDC)) = −0.020 × (CDC reference value) + 1.112 [n: 280, R^2 = 0.063 (p-value: <0.001)]. p-value and 95% CI are <0.001 and (−0.029, −0.011) for slope, respectively. p-value and 95% CI are <0.001 and (0.671, 1.553) for intercept, respectively. (C) CDC: US Centers for Disease Control and Prevention. LDL-C: Low-density lipoprotein cholesterol. x-axis indicates CDC reference value of LDL-C (unit: mg/dL) in the concentration range from 71.5 to 173.3 mg/dL and y-axis indicates the LDL-C bias between Osaka and CDC (unit: mg/dL). y (bias (Osaka–CDC)) = −0.013 × (CDC reference value) + 1.186 [n: 280, R^2 = 0.059 (p-value: <0.001)]. p-value and 95% CI are <0.001 and (−0.020, −0.007) for slope, respectively. p-value and 95% CI are 0.004 and (0.376, 1.996) for intercept, respectively.

2.5. Statistical analysis

We used protocol EP9-A from the Clinical and Laboratory Standards Institute [20–22] for bias estimation and STATA12 analysis program for all other calculations.

3. Results

The concentration ranges of the 67 lots used in the CRMLN surveys were 122.3–223.7 mg/dL, 27.0–72.4 mg/dL, and 71.5–173.3 mg/dL for BFC, HDL-C, and LDL-C, respectively. For 15 years, the reference laboratory at Osaka meets CRMLN accuracy and precision performance goals for BFC, HDL-C and LDL-C (Table 2).

The mean percent bias between the Osaka laboratory and the CDC reference laboratory was <0.5% for all analytes, with limits of agreement being very narrow. Bias and regression analyses show that the bias, though small, is significant. The observed bias is well-below the allowable bias for CRMLN laboratories. The individual sample biases at low analyte concentrations tend to be positive, and at high concentration the biases are negative for all analytes (Fig. 1A–C).

From the estimation by regression line, the absolute bias between CDC and Osaka in the clinical decision levels was estimated as 0.40 mg/dL for BFC at 180 mg/dL, 0.32 mg/dL for HDL-C at 40 mg/dL and 0.62 mg/dL for LDL-C at 140 mg/dL. The bias was small, but the mean value of absolute bias in upper 10% and lower 10% concentration of reference value was larger than that in middle 80% for BFC (1.45 mg/dL vs. 0.98 mg/dL: p = 0.01). There was no difference of bias related to concentration for HDL-C (0.69 mg/dL vs. 0.54 mg/dL: p = 0.19) and LDL-C (1.04 mg/dL vs. 1.10 mg/dL: p = 0.70) (Table 3).

Assessing measurement bias over time showed no significant trend from May 1997 to October 2012. This is indicated in no significant bias observed with lot bq47, which was analyzed quarterly over 2.5 years. Furthermore, no significant trend in measurement bias was observed for this period (Fig. 2).

Correlation plots between BFC (x-axis, unit: %bias vs. CDC) and LDL-C (y-axis, unit: %bias vs. CDC) of the Osaka laboratory are positively correlated (y = 1.088x − 0.208, n = 280, R^2 = 0.652 (p-value < 0.001), p-value and 95% CI for slope are <0.001 and (0.994, 1.182), respectively, p-value and 95% CI for intercept are <0.001 and (−0.289, −0.128), respectively) (Fig. 3D). In contrast, only weak correlations are observed between the biases from BFC (x-axis, unit: %bias vs. CDC) and HDL-C (y-axis, unit: %bias vs. CDC). (y = 0.480x + 0.513, n = 280, R^2 = 0.057 (p-value < 0.001)) (Fig. 3E). Similarly, only weak correlations existed between the biases from LDL-C (x-axis, unit: %bias vs. CDC) and HDL-C (y-axis, unit: %bias vs. CDC). (y = −0.441x + 0.299, n = 280, R^2 = 0.087 (p-value < 0.001)) (Fig. 3F).

4. Discussion

LDL-C is a key biomarker for cardiovascular disease risk assessment, and it is the primary target for treatment. No RMP currently exists for direct measurement of LDL-C. Therefore, the BQ approach was established to assign LDL-C reference values to serum materials. Like all RMPs, it is not intended for use in patient care because of its technical demands (e.g. overnight UC, manual volumetric sampling, and reconstitution of the bottom fractions) [23,24]. However, the technical limitations of this method such as sample throughput or complexity are similar to those of other RMPs [25]. Because measurement results are traceable to an RMP and the International System of Units, it is important to assure that this method is highly reproducible and accurate over time. Efforts by CDC and its partners to assure the accuracy of LDL-C measurements have been ongoing for over 15 years. The CRMLN assures the accuracy of LDL-C measurements by providing reference measurement service to the clinical laboratory community to establish metrological traceability to the CDC RMP. Only a few studies have examined the performance of BQ RMP [26–28]. This study describes the performance of LDL-C value-assignment performed in one CRMLN laboratory over 15 years.

The actual cholesterol measurements are traceable to pure compound certified reference materials and thus are traceable to SI as outlined in ISO 17511. The isolation of the lipid fractions is traceable to a RMP, which is also outlined in ISO 17511. To our knowledge, ISO 17511 does not define nor require a so called "gold standard". Because

Table 3
Comparison of absolute bias between middle 80% and upper/lower 10% of reference values.

Lipid	Range of middle 80% of reference (CDC) value	Mean of absolute bias in middle 80% of reference (CDC) value	Mean of absolute bias in upper 10% and lower 10%	p-value
BFC	132.80–214.79 mg/dL	0.98 mg/dL	1.45 mg/dL	0.01
HDL-C	33.50–64.50 mg/dL	0.54 mg/dL	0.69 mg/dL	0.19
LDL-C	95.50–165.39 mg/dL	1.10 mg/dL	1.04 mg/dL	0.70

BFC: Bottom fraction cholesterol.

cholesterol measurements are traceable to SI, we prefer to use the term "accuracy" in the manuscript. The CDC BQ RMP is classified as a higher order reference measurement procedure used to assign reference values on frozen reference materials. The CDC LDL-C RMP is the reference point for LDL-C recommended by the NCEP Lipoprotein Measurement Working Group. The accuracy reported in the paper refers to the accuracy compared to the CDC LDL reference values. The CRMLN laboratories achieve traceability to CDC RMP through monitoring.

The BQ method combines the removal of triglyceride (TG)-rich VLDL by UC, isolation of HDL from the UC bottom fraction, and cholesterol analysis of the bottom fraction and HDL supernatant. Therefore, the performance of HDL-C and BFC measurements needs to be considered when assessing factors affecting LDL-C target value assignments.

Over 15 years, the BQ RMP operated at the Osaka laboratory provided highly accurate and precise measurements of HDL-C and LDL-C, as indicated in the high agreement with the CDC reference laboratory. The observed mean bias is well within the allowable bias for CRMLN laboratories. The CRMLN focuses mainly on assuring accuracy of measurements around the clinical decision levels, which would be 40–60 mg/dL for HDL-C and 100–160 mg/dL for LDL-C (Fig. 1B,C); most of the serum pools used in CRMLN cover these ranges. Within these ranges, no significant mean bias and no proportional bias between the 2 methods were observed (Table 3). Considering that the LDL-C value assignments are derived from two separate measurements and that this RMP is technically very demanding, the overall performance and performance over time is remarkable. The data demonstrate that this method can be operated in a highly precise manner over long periods of time.

The CDC BQ method has been accepted as the most reliable RMP for HDL-C and LDL-C measurements, and it was recommended by the NCEP as the RMP method for HDL-C and LDL-C. The BQ method was used to establish the concentrations of the major lipoprotein classes in almost all epidemiological studies and clinical trials on which current guidelines for CVD risk assessment are based. It is used in the assignment of LDL-C reference values to calibrators or standards, patient specimens or bench-level quality control materials, and in the evaluation of direct [29,30] and homogeneous methods [31–33]. In the "Program Recommendations for the Measurement of Low-Density Lipoprotein Cholesterol: Executive Summary" [16], Bachorik et al. encouraged the early development of homogeneous methods and suggested that new methods for measuring LDL-C should be developed that are capable of directly quantifying LDL-C, and which should not be based on calculations of the difference between two or more measured values. The developed homogeneous methods have some advantages, such as the direct measurement of LDL-C by automated analytical instruments and possible use of non-fasting samples. However, they do have limitations [31–33]. Therefore, the BQ method is needed to assure accurate patient data that can be compared to current clinical decision points.

The reference values obtained with the BQ approach are based on the density of lipoprotein particles and their separation using specific UC conditions. LDL is not a unique molecular species; it consists of a group of similar, mixed, and atherogenic lipoproteins that vary to some degree in their chemical composition and physico-chemical particles [34]. The bottom fraction contains minor, but atherogenic lipoprotein classes such as IDL and Lp(a) [17,35,36]. In normal individuals, both lipoprotein classes can be expected to contribute 2–4 mg/dL, on average, to the total cholesterol measurement; however, their concentrations may be higher in patients with CHD and in patients at risk of developing CHD by virtue of dyslipidemia. The alterations of these lipid classes can affect cardiovascular disease risk, which may not be adequately detected by the BQ approach. Therefore, new approaches, such as measurement of LDL particle numbers, have been suggested to better assess cardiovascular risk in patients with such conditions [37]. The limitation of the BQ approach needs to be considered when using this RMP for reference value assignments.

The strong correlation between the BFC bias and the LDL-C bias, as well as the weak correlation between the LDL-C bias and HDL-C bias, suggests that the accuracy of LDL-C performed is directly affected by the accuracy of the BFC measurement and, to a much lesser extent, by the HDL-C measurement. This is expected because the LDL-C is calculated from the BFC, while HDL-C is an independent measurement. Because of the good agreement between CDC RMP and Osaka RMP, the different UC conditions used by these laboratories do not appear to have a profound effect on the mean bias or individual sample biases.

In conclusion, this study demonstrates that accurate measurement of BFC is critical for LDL-C value assignment. The BQ RMP performed at the Osaka laboratory is accurate and consistent over time. This assures that calibrations of assays used in patient care are accurate, and that measurements performed in patient care meet established performance criteria. Thus, the BQ RMP ensures that current guidelines using LDL-C levels for CVD risk assessments can be applied correctly and consistently.

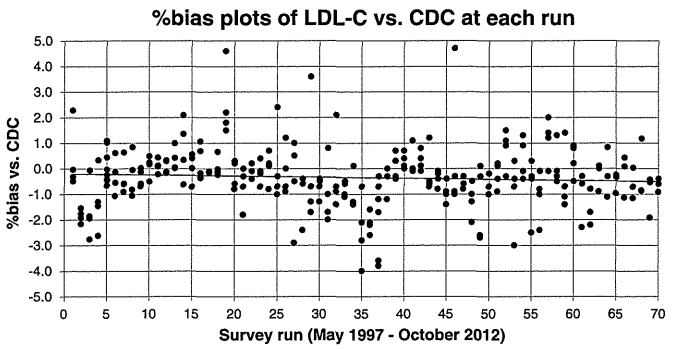

Fig. 2. %Bias plots of LDL-C vs. CDC at each survey run. CDC: US Centers for Disease Control and Prevention. LDL-C: Low-density lipoprotein cholesterol. x-axis indicates survey run number during May 1997 and October 2012 with 70 runs and y-axis indicates %bias of LDL-C vs. CDC. The accuracy criteria of %bias plots of LDL-C is ±2% of CDC reference value. Each survey run consists of 3 to 5 CDC pools for beta quantification analysis.

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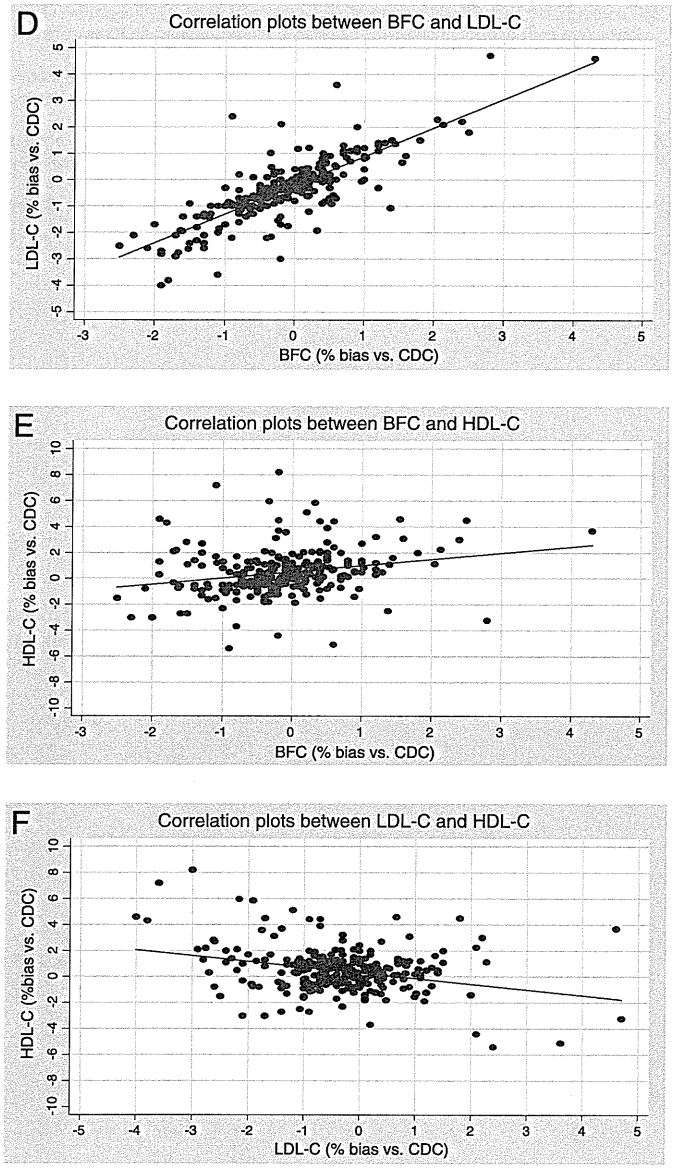

Fig. 3. Scatter plots of correlation and regression at Osaka between BFC and LDL-C (D), BFC and HDL-C (E), and LDL-C and HDL-C (F). (D) CDC: US Centers for Disease Control and Prevention. BFC: Bottom fraction cholesterol. LDL-C: Low-density lipoprotein cholesterol. CI: Confidence interval. x-axis indicates Osaka BFC (unit: %bias vs. CDC) and y-axis indicates Osaka LDL-C (unit: %bias vs. CDC). y (Osaka LDL-C) = 1.088 × (Osaka BFC) − 0.208 [n: 280, R^2 = 0.652 (p-value: <0.001)]. p-value and 95% CI are <0.001 and (0.994, 1.182) for slope, respectively. p-value and 95% CI are <0.001 and (−0.289, −0.128) for intercept, respectively. (E) CDC: US Centers for Disease Control and Prevention. BFC: Bottom fraction cholesterol. HDL-C: High-density lipoprotein cholesterol. CI: Confidence interval. x-axis indicates Osaka BFC (unit: %bias vs. CDC) and y-axis indicates Osaka HDL-C (unit: %bias vs. CDC). y (Osaka HDL-C) = 0.480 × (Osaka BFC) + 0.513 [n: 280, R^2 = 0.057 (p-value: <0.001)]. p-value and 95% CI are <0.001 and (0.250, 0.711) for slope, respectively. p-value and 95% CI are <0.001 and (0.316, 0.710) for intercept, respectively. (F) CDC: US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. LDL-C: Low-density lipoprotein cholesterol. CI: Confidence interval. x-axis indicates Osaka LDL-C (unit: %bias vs. CDC) and y-axis indicates Osaka HDL-C (unit: %bias vs. CDC). y (Osaka HDL-C) = −0.441 × (Osaka LDL-C) + 0.299 [n: 280, R^2 = 0.087 (p-value: <0.001)]. p-value and 95% CI are <0.001 and (−0.609, −0.273) for slope, respectively. p-value and 95% CI are 0.004 and (0.098, 0.499) for intercept, respectively.

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

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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.

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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. $MgCl_2$•$6H_2O$ (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, $R^2 = 0.005$].

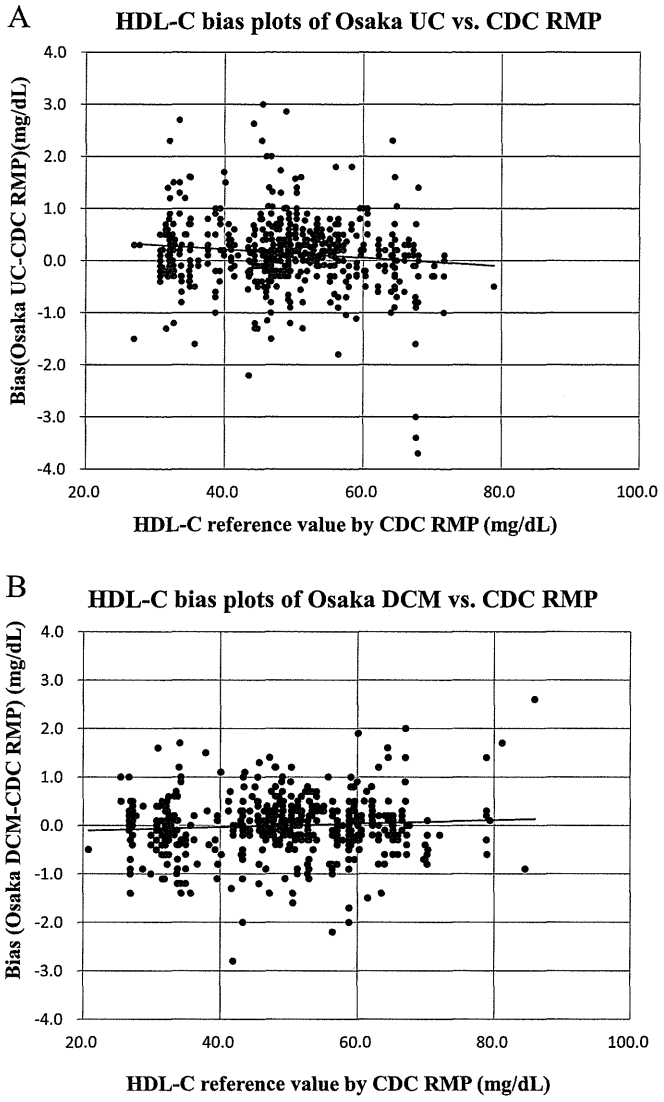
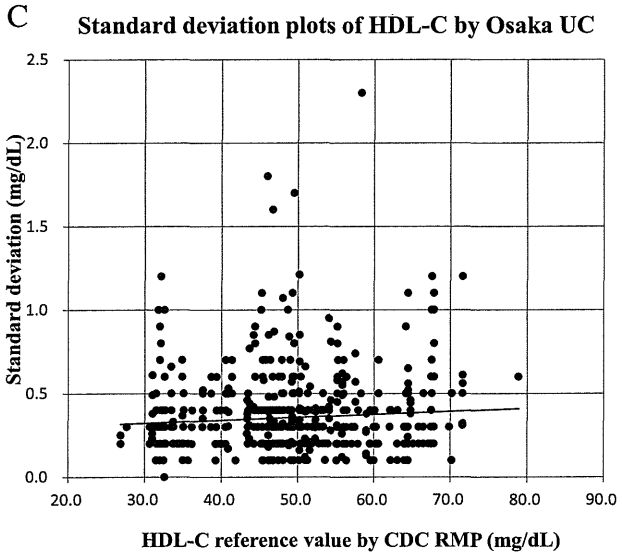


Fig. 1. A: HDL-C bias plots of Osaka UC vs. CDC RMP. The y-axis indicates the bias (mg/dl) of Osaka UC compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein cholesterol. UC: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



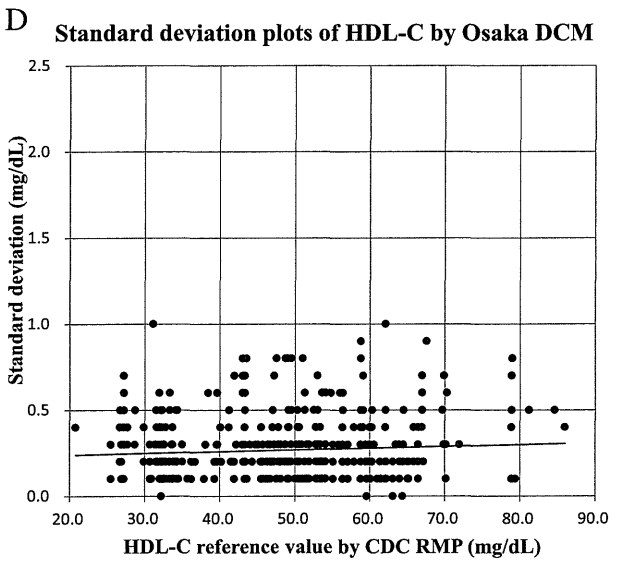


Fig. 2. C: Standard deviation plots of HDL-C by Osaka UC. The y-axis indicates the SD (mg/dl) of the Osaka UC method compared to the CDC reference value and the x-axis indicates the CDC RMP HDL-C reference value. CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density lipoprotein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

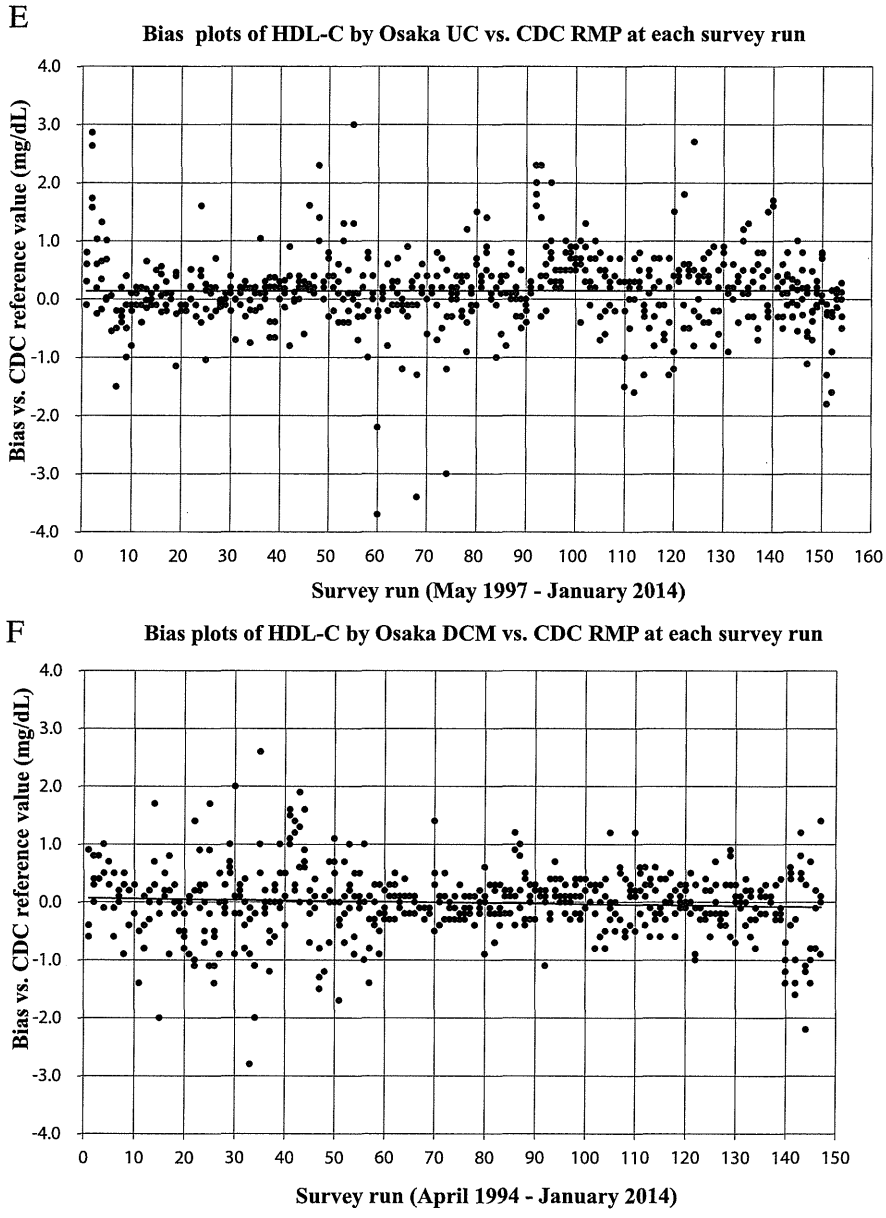


Fig. 3. E: Bias plots of HDL-C by Osaka UC vs. CDC RMP at each survey run. The y-axis indicates the bias (mg/dl) of Osaka UC compared to the CDC reference value and the x-axis indicates each survey run (May 1997–January 2014). CDC: The US Centers for Disease Control and Prevention. HDL-C: High-density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