Original Article

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

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Aim: This study was designed to clarify the current measurement performance of 7 reagent manufacturers for high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides (TG) specified for the metabolic syndrome (MetS)-focused health checkups program in Japan.
Methods: Twenty HDLC, 21 LDLC and 9 TG analytical reagent/instrument/calibrator systems (system), and combinations of reagent lots, instrument models and calibrator lots, underwent Centers for Disease Control and Prevention (CDC)/Cholesterol Reference Method Laboratory Network (CRMLN) lipid standardization. Eighty and 100% systems were requested to achieve an accuracy of within ±1% and ±2% of the reference value, so that a clinical laboratory can meet the CDC criteria.
Results: The CDC performance criteria of HDLC, LDLC and TG require an accuracy of within ±5%, ±4% and ±5%, respectively. For HDLC, all 20 systems met the criteria. Fourteen (70.0%) and 18 (90.0%) systems were within ±1% and ±2%, respectively. For LDLC, 14 (66.7%) of 21 systems met the criteria, but 7 (33.3%) failed. Five (23.8%) and 17 (81.0%) systems were within ±1% and ±2%, respectively. For TG, 8 of 9 systems met the criteria. Two (22.2%) and 4 (44.4%) systems were within ±1% and ±2%, respectively. The minimum and maximum differences of a specified sample among manufacturers were 1.6 and 11.0 mg/dL for HDLC, 7.8 and 33.0 mg/dL for LDLC, and 2.8 and 27.4 mg/dL for TG, respectively.
Conclusion: Homogeneous HDLC methods are acceptable for MetS, but further accuracy improvement of homogeneous LDLC and TG methods will be needed because of their poor performance.

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Key words; Metabolic syndrome, HDLC, LDLC, CDC/CRMLN

Introduction

The Japanese lifestyle, including eating habits, has become more westernized with marked post-war economic growth, and living and medical care levels have markedly improved, but the nation is also facing a new health problem: metabolic disorders constituting obesity, dyslipidemia, and abnormal glucose tolerance[1-3]. Moreover, dynamic changes in social and living environments have brought major quantitative increases in atherosclerotic cardiovascular diseases, malignant tumors, and their associated risk factors[4-6]. Under such circumstances, the Japanese government

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developed a standard program, The Health Checkups and Healthcare Advice with a Particular Focus on Metabolic Syndrome (MetS). In April 2008, the program enforced the provision of health checkups focusing on MetS for health-insured 40 -74 -year-old Japanese people. Three lipids, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides (TG), were measured, excluding total cholesterol.

This study was designed, (1) to clarify the current measurement performance of 7 reagent manufacturers supplying homogeneous HDLC, LDLC and TG kits, and (2) to request their 80% and 100% systems to achieve an accuracy of within ±1% and ±2% of the reference value, respectively, so that a clinical laboratory can meet the Centers for Disease Control and Prevention (CDC) criteria and join the MetS program.

Materials and Methods

Standardization Items and Participating Reagent Manufacturers

HDLC, LDLC and TG were selected for standardization. Seven Japanese reagent manufacturers participated in the CDC/Cholesterol Reference Method Laboratory Network (CRMLN) standardization program: Serotec Co., Ltd. (Hokkaido), Denka Seiken Co. Ltd. (Niigata), Sekisui Medical Co. Ltd. (Tokyo), UMA Co. Ltd. (Tokyo), Kyowa Medex Co. Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Osaka) and Sysmex Co. Ltd. (Hyogo).

Standardization Protocols for HDLC, LDLC and TG

Standardization of HDLC, LDLC and TG for the manufacturers through the CDC/CRMLN followed the HDL Cholesterol Certification Protocol[7] for Manufacturers (November, 2002), the LDL Cholesterol Certification Protocol[7] for Manufacturers (June, 2006) and the Triglyceride Certification Protocol7) for Manufacturers (October, 2003), respectively (http://www.cdc.gov/labstandards/crmln.htm); however, the TG protocol has not yet been implemented by the CRMLN and TG was not certified.

Reference Methods for Establishing the Reference Value

The reference method for HDLC is the designated comparison method (DCM). Samples were precipitated for separation using dextran sulfate (50 kDa)-Mg^{2+} reagent and HDLC in the supernatant was measured by the Abell-Kendall (AK) reference method for cholesterol. The reference method for LDLC used the beta-quantification method (BQ)[8]. The BQ method uses a three-step procedure involving ultracentrifugation, precipitation of the bottom fraction (BF) with heparin-Mn^{2+} reagent, and cholesterol quantification of both the BF and high-density-lipoproteins fractions with the AK method. Samples were given 2 spins of ultracentrifugation, and the BF cholesterol and HDLC were measured. LDLC, as defined by the National Cholesterol Education Program (NCEP), includes intermediate-density lipoprotein cholesterol (IDLC) and lipoprotein (a) (Lp(a)) cholesterol. The reference method for TG is detailed in the Procedure for the Triglyceride DCM (November, 2001). Samples were extracted with activated silicic acid and methylene chloride, and then dehydrated. The extract was dried by evaporation, hydrolyzed with KOH alcohol, and glycerol was enzymatically analyzed. Since TG DCM undergoes standardization by the CDC reference method using the chromotropic acid method, the Osaka Medical Center for Health Science and Promotion (OMC) can determine the reference value.

Samples

The standardization protocol for HDLC and LDLC allows the use of fresh, individual and preferred fasting serum or mixtures of serum from a maximum of 2 persons with 40 or more specimens. HDLC samples containing TG of 200 mg/dL or lower[9] are specified so as to include at least 5 samples each containing HDLC at concentration ranges of 20–29, 30–39, 40–49, 50–59, and 60–69 mg/dL. LDLC samples are specified so that 20% of samples each contain LDLC at 100 mg/dL or lower and 161–400 mg/dL, and 30% each contain 100–130 and 131–160 mg/dL; however, for TG, frozen samples, the same as for the LDLC measurement were used. The concentration of the internal quality control material was 30–60 mg/dL for HDLC and 130–160 mg/dL for LDLC. All specimens were collected in turn at a manufacturer and provided to both other participating manufacturers and the OMC by overnight express delivery.

Measurements

Measurements were performed in duplicate once a week at both the reagent manufacturer and OMC. The manufacturers attempted to standardize 20 systems for HDLC, 21 systems for LDLC and 9 systems for TG. A system is a combination of the reagent lot, instrument model and calibrator lot. Each clinical laboratory selects any of the systems in routine work for patients. The instruments used were model series of

Table 1. Performance Criteria for CRMLN Laboratories, Manufacturers and Clinical Laboratories

Item	Performance Criteria for CRMLN Laboratories		Performance Criteria for Manufacturers and Clinical Laboratories	
	Accuracy Criterion	Imprecision Criterion	Accuracy Criterion	Imprecision Criterion
HDLC	Bias ≤ 1 mg/dL	SD ≤ 1 mg/dL	Bias ≤ 5%	CV ≤ 4%
LDLC	Bias ≤ 2%	CV ≤ 1.5%	Bias ≤ 4%	CV ≤ 4%
TG	Bias ≤ 2.5%	CV ≤ 2.5%	Bias ≤ 5%	CV ≤ 5%

Hitachi, Toshiba, Olympus and Japan Electron Optics Laboratory (JEOL).

Performance Criteria in the CRMLN Reference Laboratory and Reagent Manufacturer

The performance criteria[10, 11] required for the lipid reference laboratory of the CRMLN, reagent manufacturers and clinical laboratories are shown by accuracy and imprecision in **Table 1**. The performance criteria of TG required for the lipid reference laboratory of the CRMLN are tentative. There are 9 grades to determine the pass or fail status for HDLC standardization: the square of the correlation coefficient, ≥0.975 [1], the %bias accuracy is ≤5% at concentrations of 40 [2] and 60 [3] mg/dL, the average relative %bias accuracy [4] and average absolute %bias accuracy [5] are ≤5%, the among-run coefficient of variation (CV) as precision is ≤4% [6], no significant difference is present at a significance level of $\alpha = 5\%$ on a t-test of bias (t-value) [7], only one within-method outlier is acceptable [8], and there are no between-method outliers [9]. There are 10 grades for LDLC standardization. the square of the correlation coefficient, ≥0.975 [1], the %bias is ≤4% at concentrations of 100 [2], 130 [3], and 160 [4] mg/dL, the average relative % bias [5] and average absolute %bias [6] are ≤4%, the among-run CV is ≤4% [7], there is no significant difference at a significance level of $\alpha = 5\%$ on a t-test of bias (t-value) [8], only one within-method outlier is acceptable [9], and there are no between-method outliers [10]. There are 4 tentative grades for TG standardization: the square of the correlation coefficient, ≥0.975 [1], the average %bias [2] and average absolute %bias [3] are ≤5%, and the among-run CV is ≤5% [4].

Statistical Analysis

The assayed results were compared using a CDC spreadsheet for HDLC and LDLC, but TG results were only evaluated statistically. The t-value is based on CV 4% and an allowable difference of 5%, assuming n-1 degrees of freedom. Among-run CV was calculated from the 20-day values of the internal quality control sample. For within-method outliers, tests of absolute and relative differences were performed. Only samples indicated by both absolute and relative tests were within-method outliers. For between-method outliers, tests of absolute and relative differences between the test method and the reference method were performed. Only samples that did not pass either test were between-method outliers.

Electrophoresis

All samples were analyzed by agarose and polyacrylamide gel electrophoresis.

Results

Measurement Performance of OMC

CRMLN lipid reference laboratories are evaluated bimonthly by the CDC. When the criteria are met, they are qualified to certify reagent manufacturers and clinical laboratories. The OMC measurement performance demonstrated that the accuracy for HDLC in March 2008 was +0.3 mg/dL in bias and the SD was 0.23 mg/dL, and the accuracy for LDLC in April 2008 was +1.6% in bias and the CV was 0.6%, while the accuracy for TG was +0.9% in bias and the CV was 0.4%. These findings were acceptable for standardization by OMC.

Measurement Performance of HDLC by Analytical Reagent/Instrument/Calibrator Systems

HDLC was measured for February 27, 2008 to April 2, 2008 with 6 different runs using 50 samples. **Table 2** shows the performance of 20 systems by the 9 grades for HDLC criteria. All systems demonstrated traceability to the HDLC accuracy base. The mean precision of all systems was 0.9% in among-run CV, and mean accuracy was 0.5% for average %bias and 2.4% for absolute %bias. The predicted bias at 40 mg/dL is at the specific medical decision point of Japan Atherosclerosis Society (JAS) Guidelines for the diagnosis and treatment of atherosclerotic cardiovascular diseases.

Table 2. HDLC Performance of 20 Analytical Reagent/Instrument/Calibrator Systems by 9 Grades

Analytical system	[1] R-square	[2] Predicted at 40 mg/dL		[3] Predicted at 60 mg/dL		[4] Avg %bias	[5] Avg abs %bias	[6] Among-Run CV	[7] t-value	[8] Within-method Outliers	[9] Between-method Outliers
		mg/dL	%bias	mg/dL	%bias						
1	0.9818	38.9	−2.7	58.6	−2.4	−2.7	3.5	0.5	0.95	0	0
2	0.9872	41.3	3.3	61.5	2.6	3.1	3.7	0.9	1.09	1	0
3	0.9898	40.8	2.0	60.4	0.7	1.8	2.7	0.7	0.63	1	1
4	0.9924	39.8	−0.4	58.9	−1.8	−0.7	2.1	0.5	0.25	0	0
5	0.9915	40.2	0.6	59.1	−1.5	0.1	2.2	0.7	0.03	0	0
6	0.9792	39.8	−0.5	59.4	−0.9	−0.7	3.1	0.7	0.25	1	0
7	0.9911	40.4	0.9	60.5	0.8	0.8	2.4	0.9	0.28	0	0
8	0.9917	40.4	1.1	60.1	0.1	0.9	2.1	0.5	0.32	0	0
9	0.9910	40.4	1.0	60.6	1.0	0.9	2.4	0.8	0.32	0	0
10	0.9910	40.5	1.2	61.2	2.0	1.2	2.7	1.1	0.42	0	0
11	0.9940	40.3	0.8	60.2	0.3	0.7	1.9	1.0	0.25	0	0
12	0.9937	40.1	0.2	59.9	−0.2	0.0	1.7	1.4	0.00	0	0
13	0.9921	40.5	1.3	60.1	0.2	1.0	2.1	2.1	0.35	0	0
14	0.9924	40.6	1.5	60.5	0.8	1.3	2.2	0.4	0.46	0	0
15	0.9934	40.2	0.4	60.0	−0.1	0.3	2.0	0.7	0.11	0	0
16	0.9927	39.8	−0.6	59.2	−1.4	−0.8	2.1	0.9	0.28	0	0
17	0.9916	40.1	0.3	59.7	−0.5	0.1	2.1	1.5	0.03	0	0
18	0.9927	40.7	1.7	60.6	1.0	1.5	2.2	0.3	0.53	0	0
19	0.9903	40.4	1.0	59.8	−0.3	0.7	2.3	1.5	0.25	0	0
20	0.9924	40.5	1.2	59.7	−0.4	0.8	2.2	0.4	0.28	0	0

Avg %bias: Average %bias Avg abs %bias: Average absolute %bias

Measurement Performance of LDLC by Analytical Reagent/Instrument/Calibrator Systems

LDLC was measured for April 8, 2008 to May 27, 2008 with 6 different runs using 51 samples. **Table 3** shows the performance of 21 systems by the 10 grades for LDLC criteria. Seven (33.3%) of all systems were smaller than 0.975 in the r-square, indicating poor stability and reproducibility in different runs. Increased absolute %bias found in systems 2, 3 and 4 is considered to be non-specific affinity to TG-rich lipoproteins, such as intermediate-density lipoproteins, very-low-density lipoproteins and chylomicron remnant from the analysis of electrophoresis. In system 7, both average %bias and average absolute %bias were over 4%, which is considered to be poor value assignment in the calibrator. The mean precision of 14 standardized systems was 0.9% in among-run CV, mean accuracy was 1.3% for average %bias and 2.9% for absolute %bias. Three (42.9%) of 7 manufacturers and 7 (33.3%) of 21 systems failed in terms of traceability to the LDLC accuracy base. The predicted biases at 2 concentrations, 130 and 160 mg/dL, are both ends of the medical decision point, 140 mg/dL, of the JAS Guidelines.

Measurement Performance of TG by Analytical Rea gent/Instrument/Calibrator systems

Table 4 shows the performance of 9 systems by the 4 grades of TG criteria. Eight of 9 systems demonstrated traceability to the TG accuracy base, while system 5 failed in terms of average absolute %bias. The difference between the maximum and minimum average %bias reached 9.2%. The mean precision of 8 standardized systems was 0.4% in among-run CV, and mean accuracy was 1.3% for average %bias and 2.9% for average absolute %bias. Two (22.2%) and 4 (44.4%) systems achieved within ±1% and ±2% from the reference value, respectively. Systems 7 and 8 were products from the same manufacturer. System 7 was calibrated with triolein as the standard and system 8 was calibrated with glycerol. There was a 6.1% difference in accuracy between the 2 systems and it was close to the theoretical value.

Achievement Rate of Manufacturer Systems

Table 5 shows the achievement rates of systems

Table 3. LDLC Performance of 21Analytical Reagent/Instrument/Calibrator Systems by 10 Grades

Analytical system	[1] R-square	[2] Predicted at 100 mg/dL		[3] Predicted at 130 mg/dL		[4] Predicted at 160 mg/dL		[5] Avg %bias	[6] Avg abs %bias	[7] Among-run CV	[8] t-value	[9] Within-method Outliers	[10] Between-method Outliers
		mg/dL	%bias	mg/dL	%bias	mg/dL	%bias						
1	0.9853	102.7	2.7	132.3	1.8	161.8	1.1	2.0	2.9	0.7	0.88	0	0
2	0.9702	101.0	1.0	134.2	3.2	167.3	4.6	2.5	5.4	0.4	1.10	0	0
3	0.9616	100.0	0.0	132.7	2.1	165.5	3.4	1.4	5.5	0.9	0.62	0	0
4	0.9501	101.2	1.2	133.9	3.0	166.7	4.2	2.5	6.2	0.5	1.10	0	0
5	0.9836	100.8	0.8	132.2	1.7	163.6	2.2	1.4	3.1	0.5	0.62	0	0
6	0.9841	101.9	1.9	133.8	2.9	165.6	3.5	2.6	3.7	0.5	1.15	0	0
7	0.9649	108.6	8.6	139.4	7.3	170.3	6.4	7.6	7.9	1.0	3.36	1	0
8	0.9698	97.6	−2.4	128.5	−1.1	159.4	-0.4	−1.5	3.8	0.7	0.66	0	0
9	0.9701	97.7	−2.3	128.6	−1.0	159.6	-0.3	−1.4	3.8	0.9	0.62	0	0
10	0.9700	98.6	−1.4	129.7	−0.3	160.7	0.4	−0.6	3.5	0.8	0.27	0	0
11	0.9874	100.0	0.0	131.2	0.9	162.4	1.5	0.7	2.4	1.5	0.31	0	0
12	0.9878	100.9	0.9	132.2	1.7	163.6	2.3	1.5	2.8	0.8	0.66	0	0
13	0.9864	99.5	−0.5	130.3	0.2	161.1	0.7	0.0	2.4	1.5	0.00	0	0
14	0.9860	101.1	1.1	132.6	2.0	164.2	2.6	1.8	3.0	1.1	0.80	0	0
15	0.9849	100.7	0.7	132.7	2.0	164.7	2.9	1.6	3.0	1.1	0.71	0	0
16	0.9872	101.1	1.1	132.9	2.3	164.8	3.0	1.9	3.1	1.0	0.84	0	0
17	0.9821	100.2	0.2	131.8	1.4	163.5	2.2	1.1	2.9	1.6	0.49	0	0
18	0.9817	100.8	0.8	133.2	2.5	165.6	3.5	2.0	3.3	0.5	0.88	0	0
19	0.9828	101.4	1.4	131.6	1.2	161.7	1.1	1.2	2.6	1.1	0.53	0	0
20	0.9864	101.1	1.1	130.3	0.2	159.5	−0.3	0.5	2.5	0.6	0.22	0	0
21	0.9868	99.6	−0.4	129.3	−0.5	159.0	−0.6	−0.5	2.5	0.5	0.22	0	0

Avg %bias: Average %bias
Avg abs %bias: Average absolute %bias

Table 4. TG Performance of 9 Analytical Reagent/Instrument/ Calibrator Systems by 4 Grades

Analytical system	[1] R-square	[2] Avg %bias	[3] Avg abs %bias	[4] Among-Run CV
1	0.9940	4.9	4.9	0.3
2	0.9985	3.7	3.8	0.3
3	0.9985	0.0	1.3	0.3
4	0.9993	1.1	1.4	0.2
5	0.9963	4.9	5.2	0.2
6	0.9991	2.4	2.5	0.7
7	0.9988	1.8	2.4	0.6
8	0.9988	−4.3	4.5	0.7
9	0.9991	0.5	2.1	0.2

Avg %bias: Average %bias Avg abs %bias: Average absolute %bias

which met the accuracy criteria for HDLC, LDLC and TG. The pass rate for HDLC was very accurate and acceptable for MetS, but LDLC and TG were not accurate for MetS at the manufacturers' sites.

Value Differences Among Reagent Manufacturers

The minimum and maximum values for HDLC, LDLC and TG assayed in the same samples among manufacturers are shown in **Table 6**. Values were calculated based on all values assayed by the same type of instrument in all systems. These findings suggest differences among the reagent manufacturers.

Value Differences by Instrument

The minimum and maximum values for HDLC and LDLC measured in the same samples among analytical instruments are shown in **Table 7**. Values were calculated based on all values measured by the same reagent and calibrator using instruments from Hitachi, Toshiba, Olympus and JEOL. The TG values were not calculated. The difference in values among instruments suggest that it is a factor in the evaluation of HDLC and LDLC.

Table 5. Achievement Rate of Analytical Reagent/Instrument/Calibrator System met the Accuracy Criteria

Item	Analytical System	Accuracy					
		Within ± 1%	Within ± 2%	Within ± 3%	Within ± 4%	Within ± 5%	± 5% Over
HDLC	20	70.0%	90.0%	95.0%	100.0%		
LDLC	21	23.8%	81.0%	95.2%			100.0%
TG	9	22.2%	44.4%	55.6%	66.7%	100.0%	

Table 6. Minimum and maximum values in the same samples among reagent manufacturers

Item	Minimum value	Maximum value
HDLC	1.6 mg/dL	11.0 mg/dL
LDLC	7.8 mg/dL	33.0 mg/dL
TG	2.8 mg/dL	27.4 mg/dL

Table 7. Minimum and maximum values in the same samples among analytical instruments

Item	Minimum value	Maximum value
HDLC	0.0 mg/dL	4.4 mg/dL
LDLC	0.2 mg/dL	10.4 mg/dL

Discussion

The MetS-focused health checkups program indicates that "the same value is obtained at any clinical laboratory in the same patient when the reference material is used". For this purpose, we requested that the achievement rate of systems at the manufacturer level should be at least 80% for within ±1% and 100% for within ±2% from the reference value. Otherwise, accuracy control in clinical laboratories adopting a heterogeneous system in which they can freely select and change reagents, instruments, calibrators or analytical parameters will be impossible. We assumed that this requirement is achievable because all manufacturers had previously undergone standardization once every two years 5-6 times over 10 years. Seven manufacturers demonstrated traceability to the HDLC accuracy base using healthy fresh samples, which did not contradict the results of the past 6 standardizations conducted from 1996 to 2006. Based on these findings, we found that HDLC can be adopted for the MetS-focused health checkups program. However, we think that there are two potential difficulties in standardizing homogeneous methods. First is the bias associated with the different assay principles of the various methods. Absorbance from the LDLC and very-low-density lipoprotein cholesterol (VLDLC) is differentiated from the absorbance used to quantify HDLC, resulting in different correlation relationships with the reference method. Second is the altered matrix characteristics of calibrators and controls[12]. The only reliable approach to establishing appropriate calibration for acceptable accuracy must be based on comparison studies conducted with fresh specimens. Calibrators must be assigned set points that result in accurate results on fresh patient samples. Regarding the variation in HDLC values among manufacturers, the minimum and maximum differences were 1.6 and 11.0 mg/dL, respectively, showing a comparatively larger bias than expected. This variation should be considered when evaluating HDLC values in clinical trials and epidemiological studies[13-15]. Only 4 companies established traceability to the LDLC accuracy base and 3 companies failed. Five (23.8%) systems achieved within ±1% and 17 (81.0%) systems achieved within ±2% of the reference value. Failures in terms of accuracy suggest a problem in value assignment in the calibrator or setting parameters in the analytical instrument. The CRMLN reference laboratory can assist in the value assignment of calibrators and control of manufacturers and in the performance of LDLC standardization every year[16]. Failures in the correlation coefficient suggested a problem regarding reproducibility among runs. Care will be necessary to incorporate LDLC into the MetS-focused health checkups[17]. After clarifying these problems, the 3 failed companies underwent re-standardization for about one month from November to December 2008, and all met the LDLC requirement. Considering the measurement limit of TG, the merit of a homogenous LDLC method is greater than Friedewald's calculation equation. Regarding the variation in LDLC values among manufacturers, the minimum and maximum differences were 7.8 and 33.0 mg/dL, respectively, showing a large bias. Eight systems for TG met the precision and accuracy criteria; however, the large bias suggested an accuracy problem in TG. Caution is necessary when incorporating TG into MetS-focused

health checkups. Additionally, for the TG calibrator, conversions to triolein and glycerol is used, i.e., double standards, in clinical laboratories. Theoretically, there should be an 8% difference between the 2 converted values. Variation in TG tended to be overlooked, but confirmation of the conversion method is necessary to compare the values among studies and laboratories. To further improve TG accuracy, new reference methods using isotope dilution/gas chromatography/mass spectrometry are being established in CDC and OMC. Iso *et al.* reported that nonfasting serum TG levels predicted the incidence of coronary heart disease among Japanese 4,452 men and 6,616 women aged 40–69 years in a 15.5-year prospective study ending in 1997[18]. This result suggests that it is very important for TG to be measured more accurately[19, 20]. There are 3 main factors leading to variation among the values: reagent, instrument and calibrator. Since the reagent and calibrator are derived from reagent manufacturers, many standardization problems are attributed to reagent manufacturers and variations due to the instruments are overlooked in many cases. We found differences in HDLC and LDLC among 4 instruments in systems using the same reagent and calibrator. The maximum difference accounted for 40% of the variation among instrument manufacturers in HDLC and 32% in LDLC. Based on these findings, not only variation among reagent manufacturers, but also among instrument manufacturers, should be considered when evaluating results. According to the lipid matrices of samples confirmed by the two electrophoresis methods, 80% of all samples used for HDLC and LDLC were healthy with regard to the electrophoresis pattern, while the remaining 20% did not show sufficient morbidity to be regarded as dyslipidemia, although a high VLDL-TG level or mid-band was noted. Samples with biases of within ± 1% and more than ± 1% from the reference value were compared. No marked difference was noted in HDLC between groups, but the presence or absence of a mid-band and the VLDLC level may have affected the degree of bias. The high-performance liquid chromatography (HPLC) method based on particle sizes can give useful qualitative and quantitative information about abnormal lipoproteins[21]. We examined the assessment of between-instrument variations in the HPLC method for serum lipoproteins and reported good traceablity to CDC reference methods for total cholesterol and HDLC[22]. We reported several discrepancies in LDLC levels using the HPLC method and the CDC reference method using lipoprotein abnormalities, such as lipoprotein lipase deficiency, E2/2 type Ⅲ hyperlipidemia, cholesteryl ester transfer protein deficiency and hyper Lp(a) lipoproteinemia[23, 24]. Another HPLC method with anion-exchange chromatography has been developed by Hirowatari *et al.*[25] who reported large discrepancies in LDLC values between anion-exchange HPLC and a homogeneous assay for cholestasis patients, including lipoprotein X[26].

In conclusion, we clarified the current measurement performance of HDLC, LDLC and TG in systems at the manufacturer level specified for a MetS-focused health checkups program in Japan. The results suggest: (1) homogeneous HDLC methods can be adopted in the program because of high accuracy, but (2) homogeneous LDLC methods are still poor in terms of both accuracy and stability, and (3) TG methods require further improvement in accuracy. We demonstrated that LDLC accuracy can be improved by re-evaluation of traceability to the BQ method through a fresh split sample comparison by value assignment of the calibrator and control serum.

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