〈Original Article〉

Clinical sample comparison of eight homogeneous assay kits for high density lipoprotein cholesterol and low density lipoprotein cholesterol

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Summary　Homogeneous assay to measure the cholesterol associated with high- density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) are widely used in clinical laboratories all over the world.　However, few reports have been published regarding the commutability of these tests for HDL-C and LDL-C.　The aim of this study was to clarify the commutability of 8 homogeneous reagents currently used to measure HDL-C and LDL-C.

The maximum coefficient of variation (CV) of the 990 health checkup samples measured with the 8 kits was 14.5% for HDL-C, whereas it was 26.9% for LDL-C. Of the 224 patient samples, high serum bilirubin levels affected the HDL-C values measured by one of the kits.　High serum bilirubin levels and the IDL ratio affected the LDL-C values measured by some kits.　Successive measurement revealed that the differences in the HDL-C and LDL-C values between laboratories were dependent on the difference in reactivity to lipoproteins.　The homogeneous LDL-C measurements were significantly correlated with the Friedewald concentrations.

1. Introduction

High-density lipoprotein (HDL) comprises a heterogeneous population of protein-rich particles that are the densest and smallest of human plasma lipoproteins. With increasing awareness of the protective role of HDL in atherosclerosis, focus has shifted to the development of rapid, specific, and reliable methods for the measurement of HDL-C, and this focus has been accelerated by the inclusion of HDL-C evaluation in international guidelines for primary and secondary prevention of coronary heart disease.

Several direct methods for the measurement of HDL-C have been introduced that are readily

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adaptable to automation[1, 2]. These methods are now widely used in clinical laboratories. However, some homogeneous methods have been reported to overestimate HDL-C values[3]. Conversely, in liver cirrhosis, a condition associated with significant alteration in lipoprotein structure and composition, homogeneous methods underestimated the HDL-C concentration[4, 5].

Low-density lipoprotein (LDL) comprises a heterogeneous population of cholesterol-rich particles that are the second densest and smallest of human plasma lipoproteins after those of HDL. In large arteries, LDL can become trapped and oxidized in the intima. Oxidation of LDL renders it recognizable by macrophages as a pathogen, resulting in endocytosis and formation of foam cells. Thus begins atherosclerosis. Numerous clinical studies have shown an independent relationship between increases in LDL-C concentrations and risk of coronary heart disease[6, 7].

In recent years, convenient homogeneous assay for LDL-C that show less influence from triglycerides (TG) have been developed and they have become widely used in clinics and for health check-ups, especially in Japan[8, 9]. However, because different homogeneous assays for LDL-C employ different measurement principles, discrepancies have been reported with intermediate-density lipoprotein (IDL), lipoprotein(a), and abnormal lipoproteins associated with liver dysfunction[10-12]. HDL-C and LDL-C homogeneous methods failed to meet the National Cholesterol Education Program total error goals for diseased individuals[13].

In the current study, we used health checkup and patient samples to compare the measurement commutability of 8 kinds of homogeneous reagents for HDL-C and LDL-C.

2. Subjects and Methods

1. Subjects

Fresh serum samples were obtained from 990 participants who underwent health checkups during February 2008. Serum samples from 224 patients with metabolic disease were obtained and divided into 6 groups: group 1: total cholesterol (T-CHO), 108-231 mg/dl and TG , 47-148 mg/dl; group 2: T-CHO, 146-368 mg/dl and TG, 33-791 mg/dl; group 3: T-CHO 209-329 mg/dl and TG 30-198 mg/dl; group 4: TG 60-1045 mg/dl; and group 5: HDL-C >101mg/dl; group 6: total bilirubin (T-Bil) >10.1mg/dl (Table 1). Data from 1570 patients, assessed for total cholesterol (TC), LDL-C, TG and HDL-C excluding high TG samples (>400mg/dL), were used for the comparison study using the Friedewald formula.

The study was approved by the ethics committee of the Medical Faculty of University of Tsukuba and conducted in accordance with the guidelines of the Declaration of Helsinki.

2. Homogeneous kits

The 8 HDL-C and LDL-C assay kits（Table 2）compared were as follows: Selective detergent method; Determiner L HDL-C/M and Determiner L

Table 1　Profiles of subject groups

Group	T-CHO	TG	HDL-C	T-Bil	Sample
1	108～231	47 ～ 148	—	—	90
2	146～368	33 ～ 791	—	—	62
3	209～329	30 ～ 198		—	12
4	189～409	60～1045	—	—	19
5	—	—	101 ↑		22
6	—	—	—	10.1 ↑	19

(Unit: mg/dl)

Table 2　Profiles of the kits

| Manufacture	Abbreviation	Type of methode	comments		Laboratory	
			HDL-C	LDL-C	HDL-C	LDL-C
Kyowa Medex	KY	Selective detergent	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)	A,D	D
Sysmex	CI	Selective detergent	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and VLDL		
Kainos	KA	Selective detergent	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)		
Serotec	SR	Selective detergent	Doesn't measure ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)		
Denka	DE	Elimination	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)		
Sekisui	SE	Elimination	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)	B,C,E,F	A,B,C,E,F
Toyobo	TO	Elimination	Uncertain whether to measure to ApoE-rich HDL	Partially reacts to IDL-C		
Wako	WA	Elimination	Partially reacts to ApoE-rich HDL	Partially reacts to IDL-C and High Lp(a)		
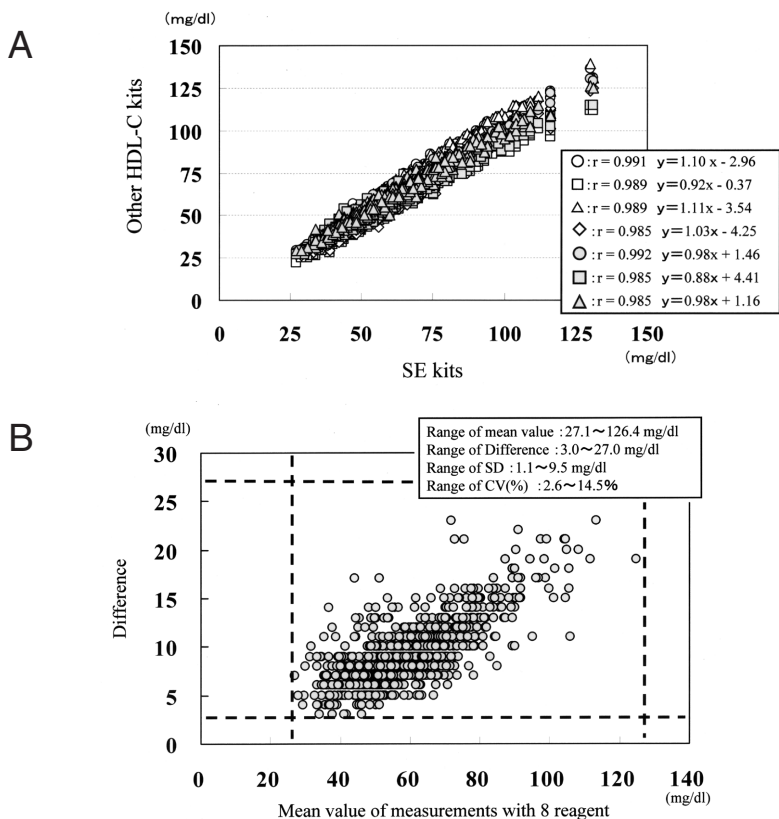
Fig. 1   A: Correlations between the SE HDL-C kit and other HDL-C kits in the health checkup samples
(n=990), B: Differences in the values of each samples measured by the 8 kits. symbols: ○, KY;
□, CI; △, KA; ◇, SR; ●, DE; ■, TO;▲ , WA.

LDL-C/M (Kyowa Medex, Tokyo, Japan), HDL-C Reagent-KL [Kokusai] and LDL-C Reagent-KL [Kokusai] (Sysmex, Hyogo, Japan), Aqua-auto Kainos HDL-C Test kit and Aqua-auto Kainos LDL-C Test kit (Kainos, Tokyo, Japan), Serotec HDL-C and Serotec LDL-C (Serotec, Hokkaido, Sapporo, Japan).

Elimination method; HDL-EX(N) and LDL-EX(N) (Denka Seiken, Niigata, Japan), Cholestest NHDL and Cholestest LDL (Sekisui Medical, Tokyo, Japan), DIACOLOR/HDL and DIACOLOR/LDL (Toyobo, Tokyo, Japan), L-Type HDL-C M(2) and L-Type LDL-C M (Wako Pure Chemical Industries, Osaka, Japan)

3. Instruments

The analyzers used for this study were the Hitachi,

7600-020E and 7180 clinical analyzers (Hitachi High Technologies, Tokyo, Japan).

4. Electrophoresis

The samples with discrepant kit measurements were analyzed by agarose gel electrophoresis. The lipoprotein bands were densitometrically determined. Cholesterol and TG were determined using a rapid electrophoresis system.

5. Comparison of measurements obtained by different laboratories

To compare the differences in the concentration measurements obtained among the 6 laboratories, we used the successive determination method as follows: The samples measured by one laboratory were
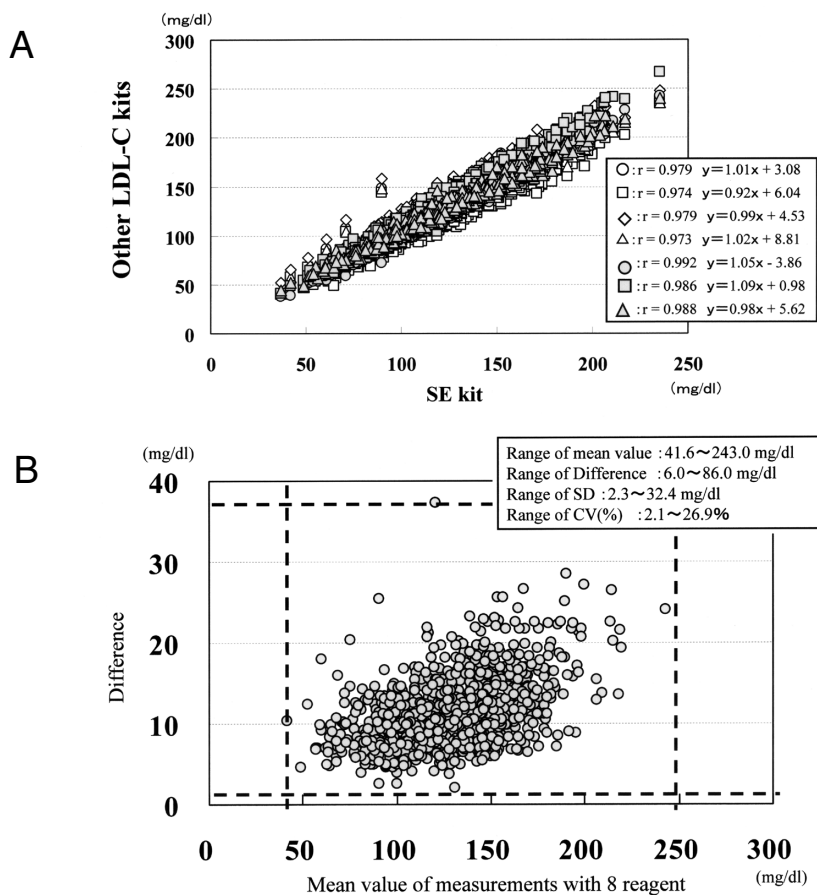
A

(mg/dl)

Other LDL-C kits

300

250

200

150

100

50

0

○：r＝0.979　y＝1.01x＋3.08
□：r＝0.974　y＝0.92x＋6.04
◇：r＝0.979　y＝0.99x＋4.53
△：r＝0.973　y＝1.02x＋8.81
○：r＝0.992　y＝1.05x − 3.86
□：r＝0.986　y＝1.09x＋0.98
△：r＝0.988　y＝0.98x＋5.62

0　　50　　100　　150　　200　　250

SE kit

(mg/dl)

B

(mg/dl)

40

30

Difference

20

10

0

Range of mean value : 41.6～243.0 mg/dl
Range of Difference : 6.0～86.0 mg/dl
Range of SD : 2.3～32.4 mg/dl
Range of CV(%) : 2.1～26.9%

0　　50　　100　　150　　200　　250　　300

Mean value of measurements with 8 reagent

(mg/dl)

Fig. 2　A: Correlations between the SE LDL-C kit and other LDL-C kits in the health checkup samples (n=990), B: Differences in the values of each samples measured by the 8 kits. symbols : ○, KY; □, CI; △, KA; ◇, SR; ●, DE; ■, TO;▲ , WA.

measured again by another laboratory on the following day, since comparison studies should be conducted with fresh specimens. In this experiment, the laboratories used the KY kit or SE kit.

6. Statistics

The correlations were estimated using Pearson's correlation coefficient and linear regression analysis. Statistical analysis was performed using Excel 2003 (Microsoft) with the Statcel 2 software plug-in with Windows XP.

3. Results

1. Comparison of the 8 kits using health check-up samples

The HDL-C concentrations of the health checkup samples measured by the 8 different types of homogeneous kits were strongly correlated (Figure 1A). Pearson's correlation coefficients (r) were 0.982-0.998. Figure 1B shows the different values for HDL-C obtained in the same samples measured by the 8 kits. The maximum CV of the 990 health checkup sample measurements was 14.5%.

The LDL-C concentrations measured by the 8 homogeneous kits were also strongly correlated (Figure 2A). Pearson's correlation coefficients were 0.964-0.998. However, the differences between the LDL-C values were much larger than between the HDL-C values. Figure 2B shows the different values

(A)

Group 1(n=90)

○	r=0.990	y=0.99x + 1.45
□	r=0.993	y=0.93x + 2.03
△	r=0.983	y=1.03x + 1.26
◇	r=0.988	y=1.03x - 1.83
○	r=0.992	y=1.01x - 1.27
□	r=0.995	y=0.91x + 3.53
△	r=0.978	y=0.92x + 3.56

(B)

Group 2(n=62)

○	r=0.996	y=1.01x - 0.42
□	r=0.994	y=0.91x + 2.28
◇	r=0.994	y=1.07x - 5.16
△	r=0.994	y=1.00x + 3.31
○	r=0.994	y=1.00x + 0.49
□	r=0.994	y=0.87x + 9.63
△	r=0.992	y=0.94x + 1.92

(C)

Group 3(n=12)

○	r=0.996	y=1.01x + 1.42
□	r=0.991	y=0.81x + 11.26
◇	r=0.978	y=0.96x + 2.42
△	r=0.986	y=0.91x + 11.76
○	r=0.984	y=0.93x + 8.33
□	r=0.994	y=0.78x + 18.14
△	r=0.983	y=0.91x + 6.63

(D)

Group 4(n=19)

○	r=0.997	y=1.02x - 1.20
□	r=0.995	y=0.95x - 1.95
◇	r=0.992	y=1.08x - 6.50
△	r=0.985	y=0.97x + 6.51
○	r=0.993	y=1.02x + 0.62
□	r=0.991	y=88x + 8.77
△	r=0.996	y=0.91x + 3.08

(E)

Group 5(n=22)

○	r=0.995	y=1.07x - 3.30
□	r=0.995	y=1.00x - 3.94
◇	r=0.993	y=1.08x - 5.80
△	r=0.992	y=1.07x + 0.37
○	r=0.995	y=1.00x + 2.37
□	r=0.992	y=0.86x + 10.24
△	r=0.989	y=0.97x + 0.40

(F)

Group 6(n=19)

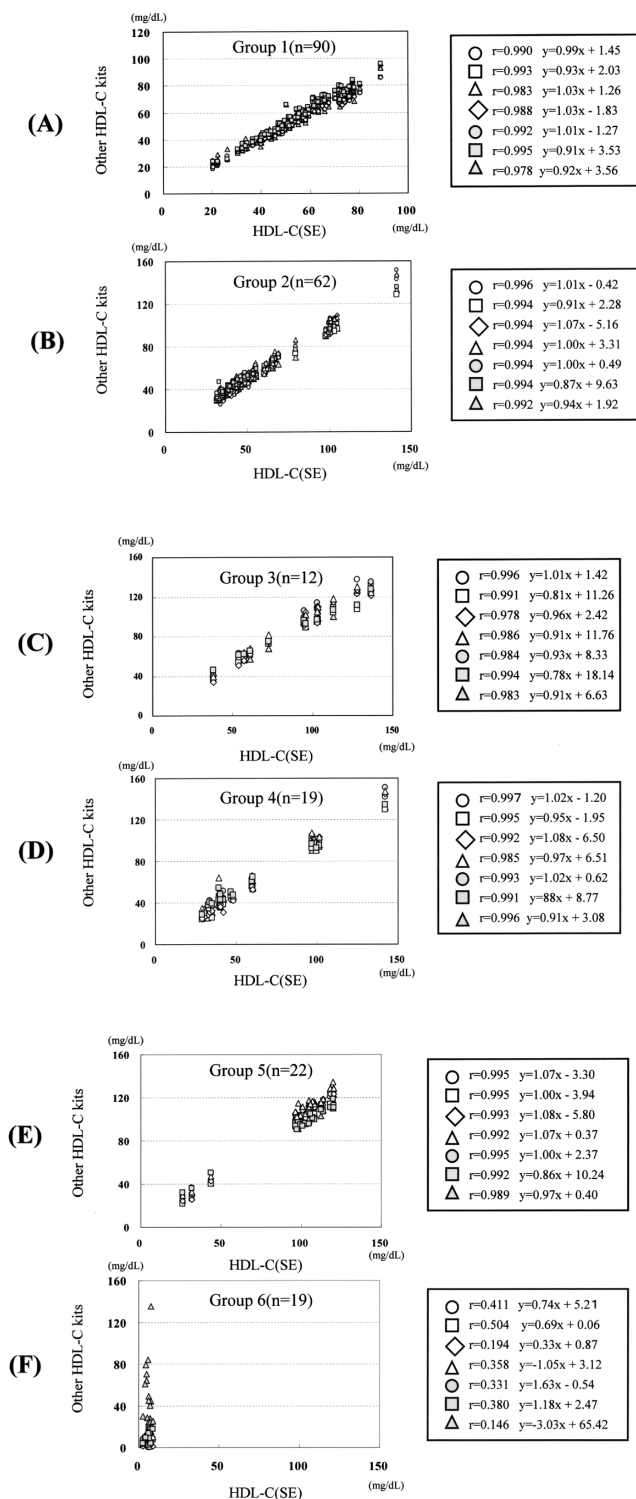|		
○	r=0.411	y=0.74x + 5.21
□	r=0.504	y=0.69x + 0.06
◇	r=0.194	y=0.33x + 0.87
△	r=0.358	y=-1.05x + 3.12
○	r=0.331	y=1.63x - 0.54
□	r=0.380	y=1.18x + 2.47
△	r=0.146	y=-3.03x + 65.42

Fig. 3 Correlations (A-F) between the SE HDL-C kit and other HDL-C kits in the patient samples of the 6 subject groups (n=225). symbols: ○, KY; □, CI; △, KA; ◇, SR; ●, DE; ■, TO; ▲, WA.
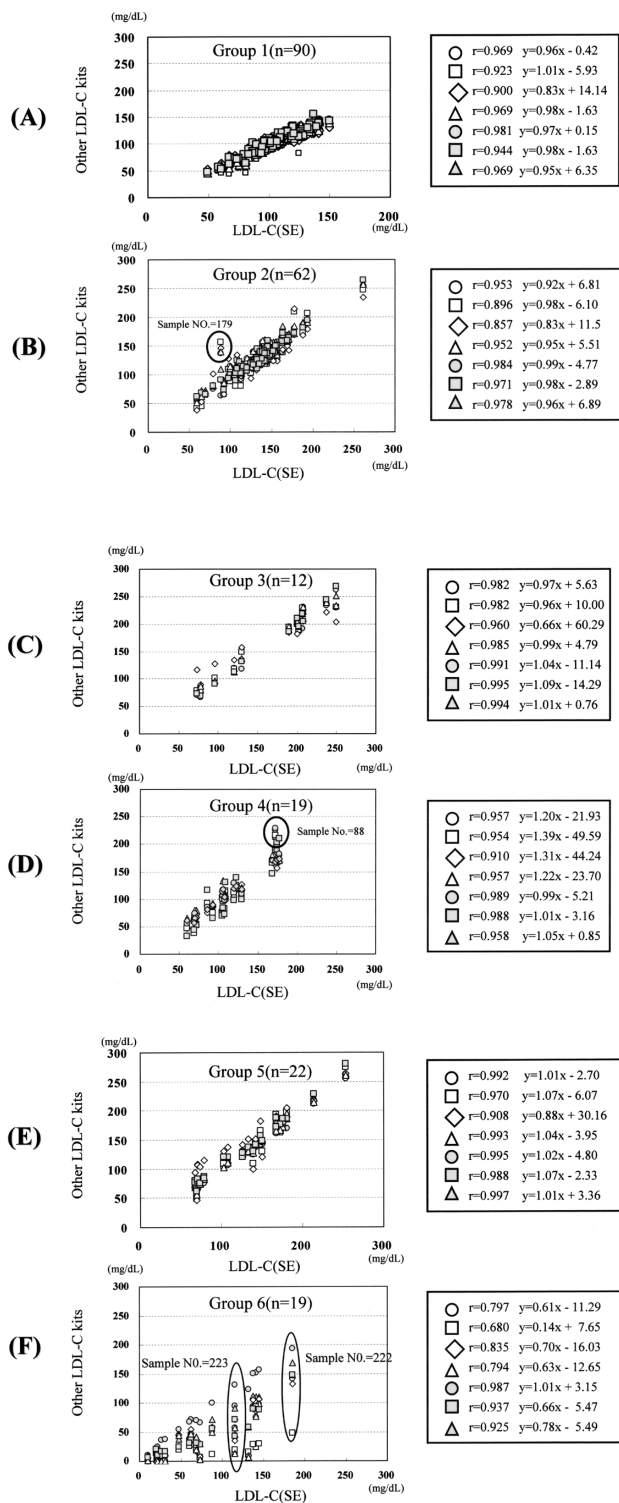
Fig. 4   Correlations (A-F) between the SE LDL-C kit and other LDL-C kits in the patient samples (n=225)
symbols: ○, KY; □, CI; △, KA; ◇, SR; ●, DE; ■, TO;▲ , WA

in the same samples measured by the 8 LDL-C kits. The maximum CV of the 8 LDL-C kits was 26.9%. The differences between the HDL-C and LDL-C values seemed to result from the different reactivities to lipoproteins of the kits (see kits profiles, Table 2).

2. Comparison of the 8 kits using patient samples

Patients were divided to 6 groups according to their lipid profiles and bilirubin levels (Table 1).

Figure 3A-F shows the correlations of the HDL-C concentrations in the samples of the 6 patient groups measured by the 8 homogeneous kits. In all but the high bilirubin group, Pearson's coefficients (r) were 0.978-0.997. As shown in Figure 3F, high serum bilirubin levels affected the HDL-C values measured by the kits, especially by the WA kit.

Figure 4A-F shows the correlations of the LDL-C concentrations in the samples of the 6 patient groups measured by the 8 homogeneous kits. The LDL-C values varied among the kits. The differences might have been due to the different reactivities to lipoproteins affected by liver disorder, as suggested by the following examination.

3. Electrophoresis analysis of the outliers

One sample from group 2, 1 sample from group 4 and 2 samples from group 6 with discrepant kit measurements were analyzed by means of agarose gel electrophoresis. We also detected cholesterol and TGs using dye.

Figure 5A shows the result of the analysis of the sample from group 6 (high bilirubin) in which a band
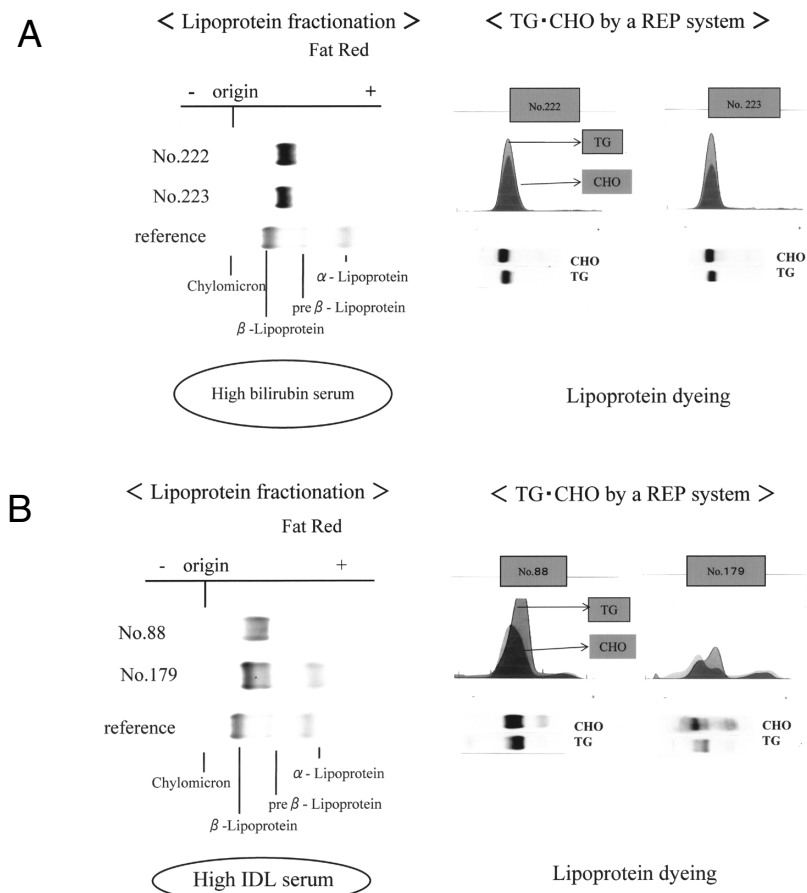

Fig. 5   Electrophoresis analysis of the outliers

was stained broadly between the pre-β and β positions in the 2 samples of group 6 such as a lipoprotein X and lipoprotein Y. TGs and cholesterol were also detected in the same position. The α-lipoprotein band was not observed at all

Figure 5B shows the result of the analysis of the sample from group 2 and 4 (high IDL) in which a band was stained broadly between pre-β and α positions such as a type Ⅲ hyperlipemia. TGs and cholesterol were also detected in the same position. These results suggest that the difference in the reactivity of the kits to lipoproteins caused the differences in the LDL-C concentrations.

4. Comparison of measurements obtained by different laboratories

We compared the difference in the patient samples of HDL-C and LDL-C concentrations measurements obtained by 6 different laboratories (laboratories A-F). As shown in Figure 6A, the differences in HDL-C concentration measurements obtained by the laboratories were due to the differences in measurement methods (selective detergent method or elimination method). Systemic difference existed in the successive measurements by laboratories C and D and by laboratories D and E. Laboratory D used the KY kit, whereas laboratories C and E used the SE kit.

As shown in Figure 6B, the differences in the LDL-C concentration measurements obtained by the laboratories were due to the differences in measurement methods. Systemic difference existed in the successive measurements obtained by laboratories C
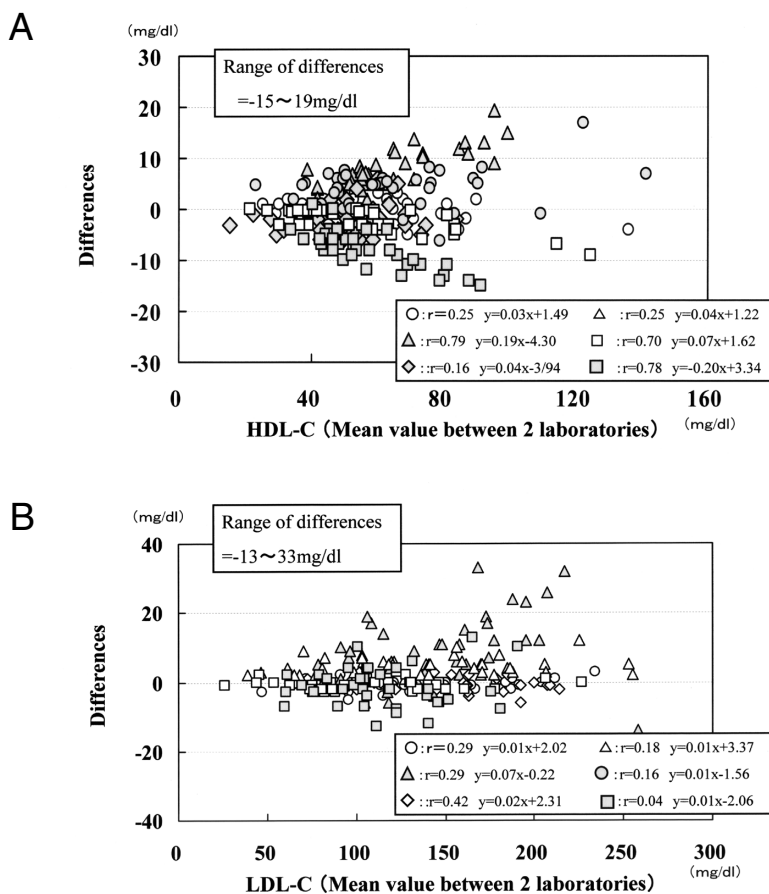

Fig. 6   Differences in HDL-C (A) and LDL-C (B) measurements obtained by successive laboratories.
symbols: ●, labs B-A; □, labs C-B; ▲, labs D-C; ■, labs E-D; ○, labs F-E; ◆, labs A-F.

and D. Only laboratory D used the KY kit. The successive determination method revealed that the differences of HDL-C and LDL-C values between laboratories depended on the difference in the reactivity of the kits to lipoproteins.

5. Comparison of the friedewald equation and a direct homogeneous assay for LDL-C

Since LDL-C has been commonly estimated using the Friedewald equation (F), we compared the values obtained by a direct homogeneous kit (SE kit) with the Friedewald equation[14].

Figure 7 shows the correlations between the LDL-C (F) and the LDL-C (SE kit) in the health checkup samples (n= 982) and the patient samples (n=1570). In these samples, the correlations between the Friedewald equation and the homogeneous LDL-C assays were as follows: LDL-C (SE kit): r = 0.965, y = 0.93x + 3.6; r = 0.946, y = 0.97x + 1.01. Figure 8 shows the differences between the LDL-C (F) and LDL-C (SE kit) in the health check up samples and the patient samples. The mean differences between the LDL-C (F) and LDL-C (SE kit) in the health up samples and the patient samples were 4.75 mg/dl and -2.14 mg/dl and the SDs were 7.97 mg/dl and 10.2 mg/dl.
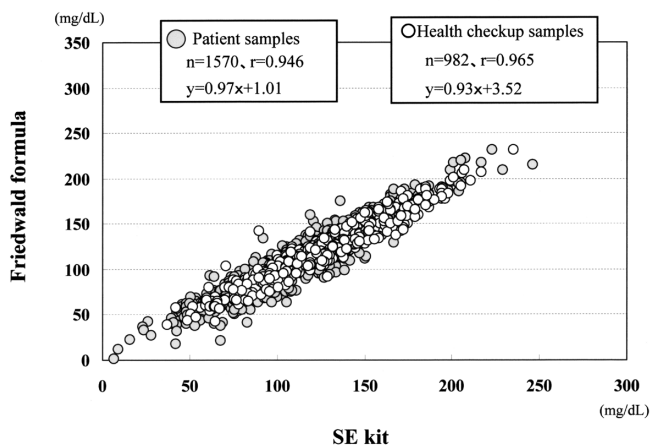

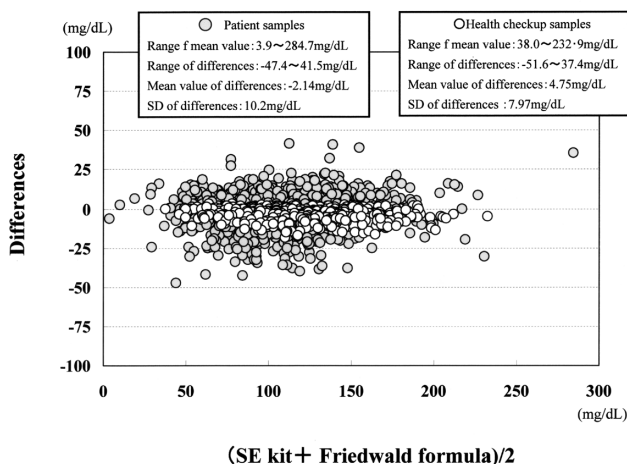Fig. 7   Correlations for LDL-C obtained by the friedewald equation and by the SE kit.

Fig. 8   Differences between the measurements for LDL-C obtained by the friedewald equation and by the SE kit.

4. Discussion

Using clinical samples, we showed that all of the 8 HDL-C homogeneous assay kits were commutable. There was no difference between the selective methods and the elimination methods. Discrepancy was only observed in the high bilirubin samples measured by one kit (WA kit). Langlois et al.[2] also concluded that HDL-C homogeneous assays are reliable and cost-effective, except when atypical lipoprotein characteristics are present.

The HDL-C homogeneous assay was developed in Japan in 1995 and since then has been used worldwide. Kurosaki et al.[15] compared 2 kits (the SE and KY kits). Saeed et al.[16] compared 3 kits by using a precipitation method and reported that the homogeneous methods showed a positive bias in type 2 diabetes, suggesting the possibility of underestimation of cardiovascular risk in patients with the disease. They also reported that a high bilirubin level (>50umol/L) influences the homogeneous HDL-C measurements. Thus, we need to consider these factors, when we use homogeneous assay for HDL-C.

We showed that the LDL-C concentration measured by homogeneous assay kits were affected by the lipoprotein levels in patient samples. Agarose gel electrophoresis revealed discrepancies in sera showing large amounts of midband. Nakamura et al.[17] reported that LDL-C needed accuracy improvement because of its poor performance. The LDL-C values in high-bilirubin serum measured by the elimination methods were higher than those by the selective methods. In high-IDL-C serum, the values measured by the selective methods were higher than those by the elimination methods. In addition to different method principle, different reactivities of the kits to lipoproteins affect the values.

In clinical laboratories, to adopt a heterogeneous system, we select and change reagents, instruments, calibrators, or analytical parameters. Therefore, these factors may affect the values. Our successive analysis demonstrated that some systemic factors including the difference of reagent caused interference in the values.

LDL-C has been commonly estimated using the Friedewald equation. We demonstrated that homogeneous LDL-C measurements had a significant correlation with LDL-C Friedewald concentrations. Previous reports also reported good correlations. Tanno, et al.[18] demonstrated that homogeneous LDL-C measurements were significantly correlated with LDL-C Friedewald concentrations. Yamashita, et al.[9] also reported that 4 homogeneous assays for LDL-C exhibited the closest correlation with the Friedewald equation.

In the present study, the difference between the Friedewald equation and the homogeneous method was larger in the patient samples than in the health checkup samples, suggesting different TG levels in the samples. Considering the measurement limit of TGs, the merit of the homogeneous LDL-C method is greater than that of the Friedewald equation.

In conclusion, we clarified the current measurement performance and commutability of HDL-C and LDL-C homogeneous assay kits.

Acknowledgments

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