UC Davis
UC Davis Previously Published Works

Title

Determination of serum lipoprotein lipase using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer

Permalink

Authors

Machida, Tetsuo
Miyashita, Kazuya
Sone, Takuya
et al.

Publication Date

DOI

Peer reviewed

CrossMark

Determination of serum lipoprotein lipase using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer

Tetsuo Machida [a,d], Kazuya Miyashita [b], Takuya Sone [c], Sayori Tanaka [c], Katsuyuki Nakajima [d], Masayuki Saito [e], Kimber Stanhope [f], Peter Havel [f], Hiroyuki Sumino [a], Masami Murakami [a,*]

[a] Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan
[b] Immuno-Biological Laboratories, Fujioka, Gunma, Japan
[c] Fujikura-Kasei, Co., Ltd, Sano, Tochigi, Japan
[d] Gunma University Graduate School of Health Sciences, Maebashi, Gunma, Japan
[e] Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo, Japan
[f] Department of Molecular Biosciences, School of Veterinary Medicine and Department of Nutrition, University of California, Davis, CA, United States

ARTICLE INFO

ABSTRACT

Background: Lipoprotein lipase (LPL) plays a central role in triglyceride-rich lipoprotein metabolism by catalyzing the hydrolysis of triglycerides. Quantification of serum LPL is useful for diagnosing lipid disorders, but there is no rapid method of measuring LPL for clinical use.
Methods: We developed a rapid and sensitive latex particle-enhanced turbidimetric immunoassay (LTIA) serum LPL using latex bead-immobilized anti-LPL monoclonal antibodies. The assay was performed on a Hitachi 7700 P analyzer and evaluated for its validity as a method of quantitating the serum LPL concentration in parallel with ELISA.
Results: Dilution tests using LTIA produced a calibration curve from 0.5 to 800 ng/ml. Within-run CV was obtained in the range of 2.2–5.5%. No interference was observed in the testing of specimens containing potentially interfering substances such as bilirubin-F and C, hemoglobin, triglycerides and rheumatoid factor. A strong correlation between LTIA and ELISA was confirmed (n = 40, r = 0.967, y=0.99x-1.86). The normal range of LPL in pre-heparin serum was 50–77 ng/ml and in post-heparin plasma 354–410 ng/ml, respectively.
Conclusion: The LTIA assay is applicable in quantitating the concentration of LPL in both pre-heparin serum and post-heparin plasma. This assay is more convenient and faster than ELISA and highly suitable for clinical routine analysis.

1. Introduction

Lipoprotein lipase (LPL) plays a major role in the metabolism and transport of lipids and lipoproteins [1,2]. It is the enzyme responsible for the hydrolysis of core triglycerides (TG) in chylomicrons (CM) and very low density lipoproteins (VLDL), producing CM remnants and VLDL remnants, respectively. Determination of LPL in plasma has typically been routinely carried out by ELISA after the intravenous injection of heparin (with its activity and concentration). However, it is also known that a comparatively high LPL concentration (ranging approximately 30–100 ng/ml in normal controls) is found in the pre-heparin serum, with an undetectable level of LPL activity, indicating that the majority of circulating LPL is catalytically inactive, but still a ligand for the receptors [3–6].

The LPL concentration and activity in the post-heparin plasma have been clinically used for the detection of LPL deficiency [2], but in general not for the diagnosis of lipid disorders or the risk of cardiovascular disease. This is because heparin injection dissociates LPL from the blood vessel endothelium, so the result does not necessarily reflect the physiological or pathophysiological concentration of circulating LPL.

An LPL-ELISA assay using specific monoclonal antibodies was reportedly developed previously by Brunzell et al. [7] and Ikeda et al. [8] for the detection of LPL in human plasma, which involved the administration of a heparin injection to the patients before the measurement of the plasma LPL concentration. Considering the assay time and the technical steps required for the quantitative measurement by ELISA, this method is not suitable for large-scale epidemiological studies or routine clinical laboratory assay.

* Corresponding author at: Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-15, Showa-machi, Maebashi, Gunma 371-8511, Japan.
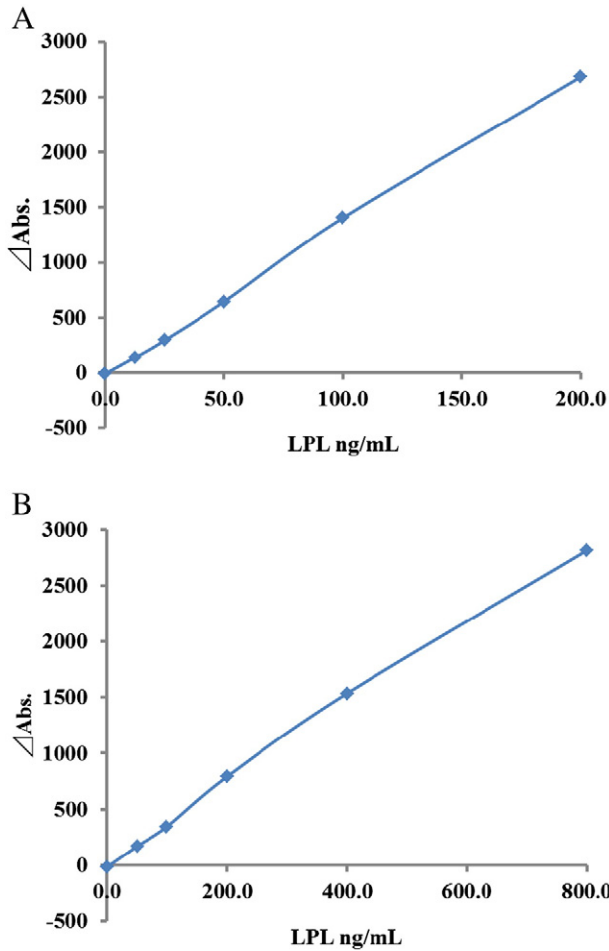E-mail address: mmurakam@gunma-u.ac.jp (M. Murakami).

Fig. 1. Linearity of LTIA. Linearity tests of LPL were performed using LTIA on a H7700 P analyzer. Two calibrators for the LPL concentrations are shown for the pre-heparin serum (Fig. 1A) and post-heparin plasma (Fig. 1B).

Therefore, there remains a need for a reliable, rapid and automated assay for the LPL concentration that has both good sensitivity and good calibrator stability, in particular if the measurement of the pre-heparin serum LPL concentration is going to be clinically meaningful and useful. LPL concentrations in the pre-heparin serum have been intensively investigated by Shirai and his colleagues the last decades using LPL-ELISA, revealing the clinical significance of the pre-heparin LPL concentration in cardiovascular and diabetic diseases [9–16].

We recently showed the possibility that the LPL concentration in the pre-heparin serum is replaceable with the LPL activity in the post-heparin plasma based on a comparison between them [17]. Therefore, the measurement of the LPL concentration in the pre-heparin serum will be able to provide more practical clinical applications in TG-rich patients without the need of a heparin injection using an automated LPL assay. As the serum pre-heparin LPL concentration is sufficiently high so as to measure it with a latex assay system, we developed a rapid and sensitive latex particle-enhanced turbidimetric immunoassay (LTIA) using latex bead-immobilized LPL-specific antibodies. The performance of the LTIA was evaluated on a Hitachi H7700 P automated analyzer. We compared its analytical properties with a commercially available ELISA assay [18] in normal volunteers, with and without heparin injection.

2. Materials and methods

2.1. Reagents

Polystyrene latex particles were obtained from Fujikura Corporation and bovine serum albumin (BSA) from Sigma, respectively. Interfering reagents, containing bilirubin F and C, hemoglobin, triglycerides and rheumatoid factor, were from Sysmex. All of the chemicals and reagents were of the highest available grade.

2.2. Preparation of blood samples

The study was conducted in relatively healthy young volunteers (some were overweight or obese) in a male (n = 19) and female (n = 21) population (Caucasian 25, Asian 5, Hispanic 4, African American 3, others 3) with a median age of 24 years and BMI of 24 at the University of California, Davis, USA. All of the volunteers were injected with heparin (50 unit/kg BW) for the LPL activity assays [19]. The University of California at Davis Institutional Review Board approved the experimental protocol and the subjects provided written informed consent to participate in the study. Two hundred forty healthy volunteers (male = 170, female = 70, median age of 26 years and median BMI of 21.6) were recruited at Tenshi College (Sapporo, Japan), with obtained written informed consent and University Ethical Committee approval [20].

The prepared serum was used for the experiments on precision, sensitivity, cross-reactivity, dilution and recovery as well as the normal range of healthy controls.

Table 1
Within-assay precision.

	Low 32.0	Mid 100.0	High 280.0
1	28.6	93.5	279.4
2	29.7	97.5	280.4
3	30.4	97.6	291.2
4	31.7	97.7	286.1
5	30.7	93.4	296.2
6	28.8	91.5	293.6
7	29.7	95.6	293.2
8	27.3	98.3	293.2
9	26.8	96.3	284.6
10	27.5	97.2	295.8
Mean (ng/ml)	29.1	95.9	289.4
S.D. (ng/ml)	1.60	2.30	6.25
CV (%)	5.5	2.4	2.2
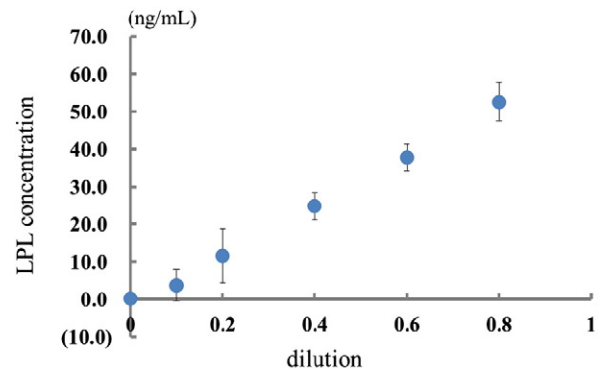
Dilution	0	0.1	0.2	0.4	0.6	0.8
Theoritical value (ng/mL)	0.0	5.4	10.7	21.4	32.2	53.6
S.D. (ng/mL)	0.35	1.64	2.77	1.42	1.39	2.01
2.6SD (ng/mL)	0.92	4.25	7.20	3.69	3.62	5.23
average (ng/ml)	0.12	3.69	11.55	24.81	37.79	52.66

Fig. 2. Analytical detection limit estimated as the concentration equal to the mean absorbance of 10 replicates of the zero calibrator plus 2.6.

2.3. Preparation of anti-LPL antibodies

The anti-LPL antibodies were raised against human recombinant LPL at Immuno-Biological Lab. The globulin fraction of 2 anti-LPL monoclonal antibodies (57A5 and 88B8) in mouse ascites was isolated using Protein-G Sepharose (GE Healthcare) and eluted with a 0.1 mol/l citrate buffer (pH 2.5), then dialyzed with PBS. The protein concentration of the antibodies was estimated by the optical density.

2.4. Preparation of the latex-immobilized antibody reagent

An optimization of the assay reagent was performed on the basis of other latex labeled immunoassays [19]. The diameter of the polystyrene latex bead and the amount of the antibody for immobilization were modified. In a preliminary study we confirmed that the particle volume has a major influence on the assay range and the amount of antibody used for immobilization contributes to the sensitivity of the assay. To adjust for the range of LPL in serum, we utilized 0.3 μm latex particles.

Polystyrene latex beads (100 mg; mean diameter 0.2 μm) were suspended in 1 ml of 0.01 mol/l HEPES buffer (pH 7.0). Nine milliliters (0.5 mg/ml) and 1 ml of 10% (wt/vol) polystyrene latex beads were incubated in 0.01 mol/l HEPES buffer (pH 7.0) for 1 h at 37 °C followed by the addition of 0.01 mol/l HEPES buffer (pH 7.0) containing 0.5% BSA at a ratio of 3 volumes to 2 volumes of latex bead suspension. After 1 h, antibody-immobilized latex beads were washed twice by centrifugation and then re-suspended in 10 ml of 0.01 mol/l HEPES buffer (pH 7.0). The suspension of the immobilized latex beads was stored at 4 °C and used as a reagent for measurement of LPL.

2.5. Calibrator preparation

In-house calibrators for LPL measurement were prepared with the recombinant LPL generated by CHO cells at IBL (Fujioka, Japan) in Tris–HCl buffer (pH 8.0). To determine the values on the in-house calibrator, calibrators from Sekisui Medical ELISA with known concentrations, were used for comparison.
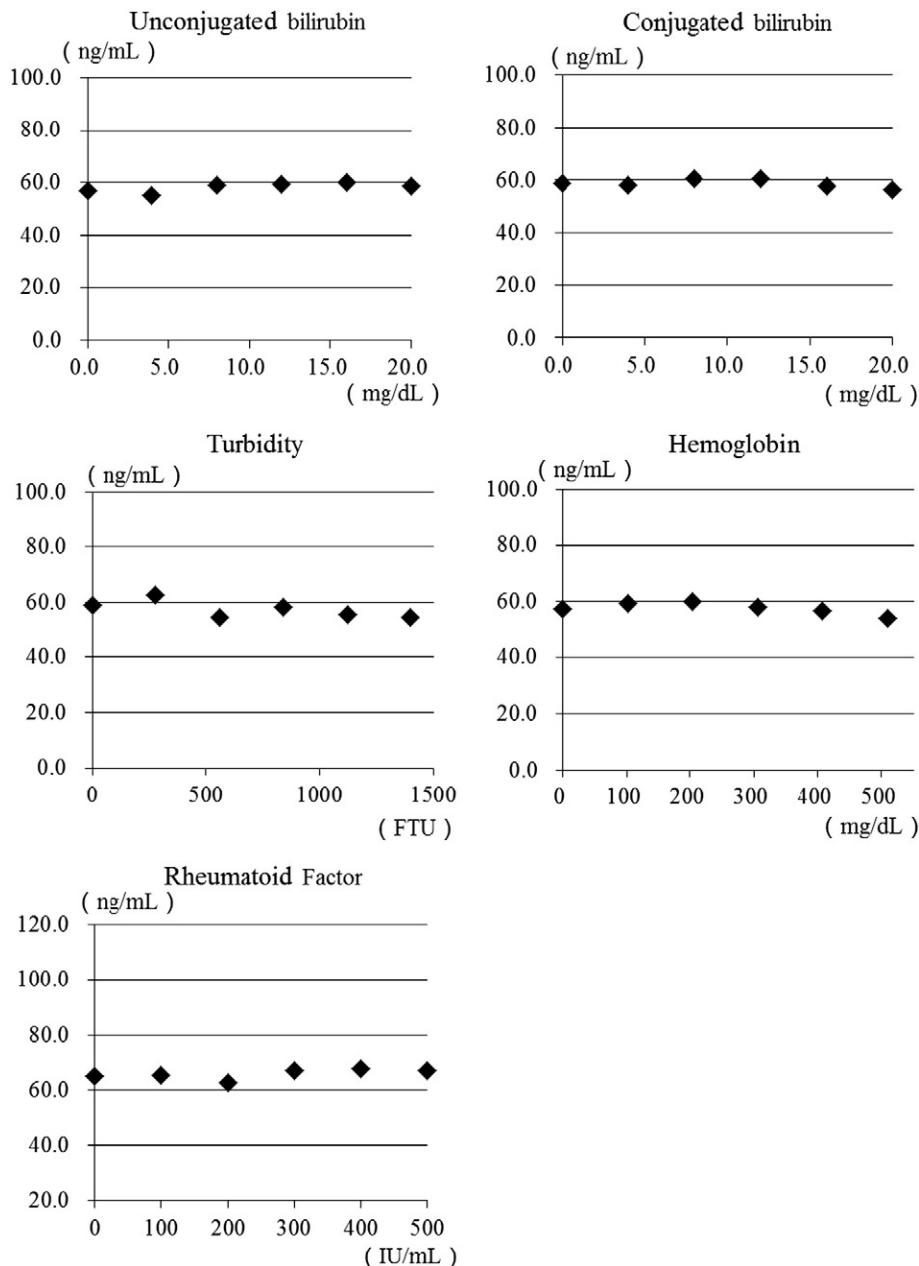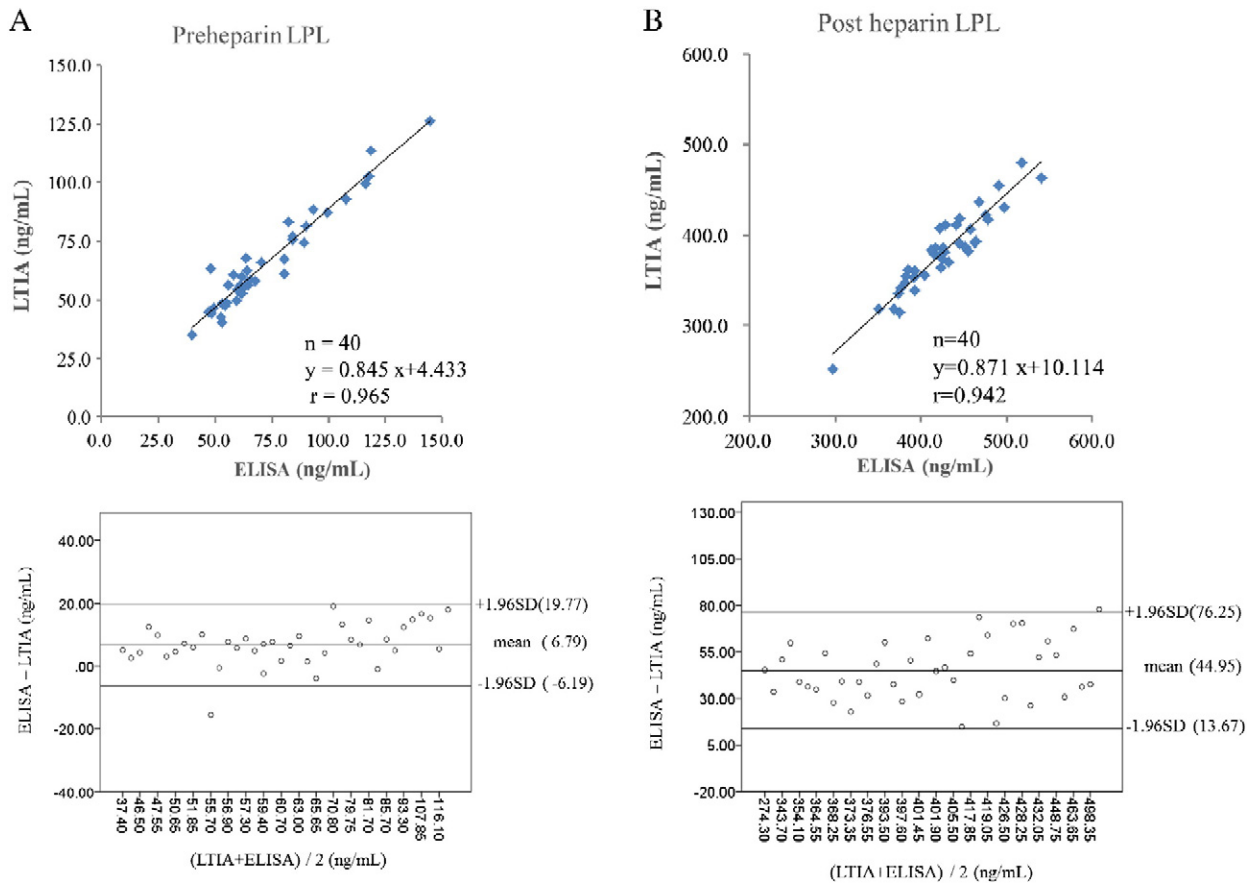

Fig. 3. Interference tests.

Fig. 4. Correlation between LTIA and ELISA for LPL. (A and B). The figure shows the results of regression analysis on the measured serum LPL concentrations between LTIA (y-axis) and ELISA (x). Fig. 4A shows the data in the lower concentration scale (<150 ng/ml) and Fig. 4B the data in the higher concentration scale (<600 ng/ml). Bland–Altman plots show the difference between the LTIA readings and ELISA readings at different ambient LPL levels at Fig. 4A &B, respectively. The x-axis shows the means of 2 readings and the y-axis shows the difference between the 2 methods. The dashed line represents the 95% confidence limits of the differences. A regression line is shown on the graph demonstrating that the mean difference between the methods is small and that there is no consistent preference between the methods.

2.6. Assay procedure

Sample processing pipetting steps and measurements were performed automatically on a Hitachi H7700 P automated analyzer (Hitachi Instruments Engineering). Briefly, 4 μl serum and 160 μl of reagent 1 (0.01 mol/l HEPES buffer (pH 7.0)) were injected into the reaction cuvette. After 5 min of incubation at 37 °C, 54 μl of reagent 2 (antibody-immobilized latex bead suspension in 0.01 mol/l HEPES buffer (pH 7.0) was added to the cuvette to start the turbidimetric immunoreaction. After another 5 min, the LPL concentration was calculated from the difference in the absorbance values between the two time points (the absorbance at 5 and 10 min) with a primary wavelength of 800 nm. The calibration curve was obtained with a series of working LPL calibrators and a six-point calibration curve was used to calculate the values of the serum samples. Measurement by ELISA was performed using an LPL ELISA kit (Sekisui Medical, Tokyo).

2.7. Statistical analysis

All analyses were executed with the Dr. SPSS II package (SPSS Inc.). The data are presented as median values, with 25th and 75th percentile values, rather than as mean values with standard deviations, because the LPL concentration was not normally distributed. Pearson's correlation coefficient was calculated between LTIA and ELISA. Univariate analysis was performed between LPL and TG, HDL-C, LDL-C, RLP-C, sd LDL-C and apolipoprotein CIII. The correlation between LPL and the above lipid

parameters was evaluated by Spearman's correlation coefficient. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Linearity

Six concentrations of recombinant LPL (0, 50, 100, 200, 400 and 800 ng/ml) were used to assess the calibration curve. After dilution (up to 10 times) with saline, serial dilutions were measured with three replicates per specimen and the linearity was evaluated. Within the measuring range of 0–200 ng/ml and 0–800 ng/ml, the deviations from theoretical values did not exceed 5%, indicating no lack of parallelism and showing good linearity (Fig. 1A and B). These results suggest a good linearity within both the low (up to 150 ng/ml) and high (up to 800 ng/ml) range.

3.2. Precision

To determine the precision of the LTIA, we performed a replication study, as shown in Table 1. Three pooled plasma samples were aliquoted into 1.5 ml plastic tubes for each concentration and frozen at −70 °C. We analyzed 3 samples in 10 runs within a day with a single calibration. As shown in Table 1, the within-run CV was 5.5% for 32 ng/ml, 2.4% for 100 ng/ml and 2.2% for 280 ng/ml.
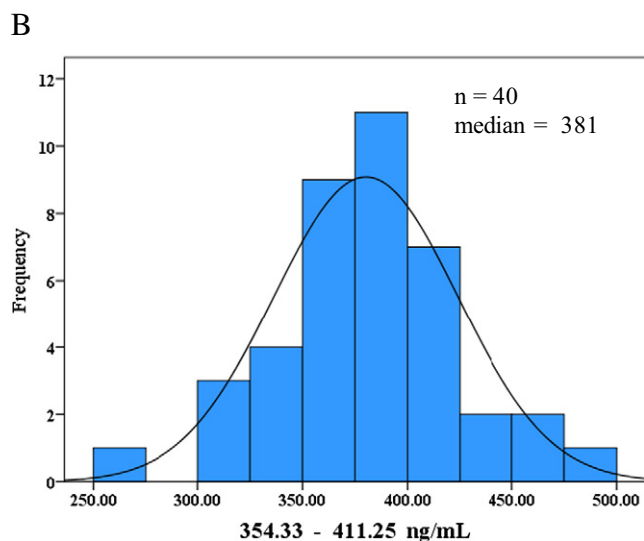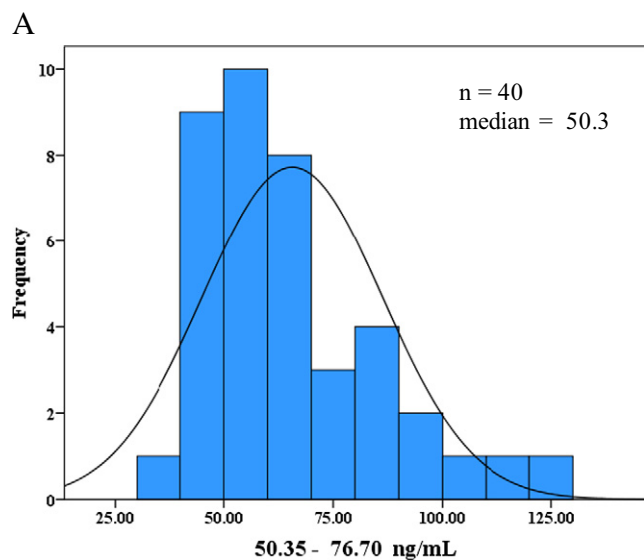
A

n = 40
median = 50.3

50.35 - 76.70 ng/mL

B

n = 40
median = 381

354.33 - 411.25 ng/mL

Fig. 5. Histogram of the LPL concentration in healthy volunteers (USA) in the pre-heparin serum (Fig. 5A) and post-heparin plasma (Fig. 5B). There was no distribution difference in the LPL concentration between males and females. The median and 25% tile 75% tile (RI; reference interval) values of the LPL concentration are shown. The post-heparin LPL concentration was significantly higher than that in the pre-heparin concentration.

3.3. Lower detection limit

Fig. 2 shows the analytical detection limit estimated as the concentration equal to the mean absorbance of 10 replicates of the zero calibrator plus 2.6 SD. The detection limit of the assay was 10.7 ng/ml

3.4. Carry-over

We measured the saline after the measurement of the samples with a high concentration of LPL (400 ng/ml). There was no detectable sign of carry-over of LPL on a Hitachi 7700 P.

3.5. Stability of LPL in plasma

There was no significant difference between the fresh samples and the conserved analytes measured after 2 days at 4 °C, or in terms of freeze thaw cycles, showing a good stability of LPL (details not shown).
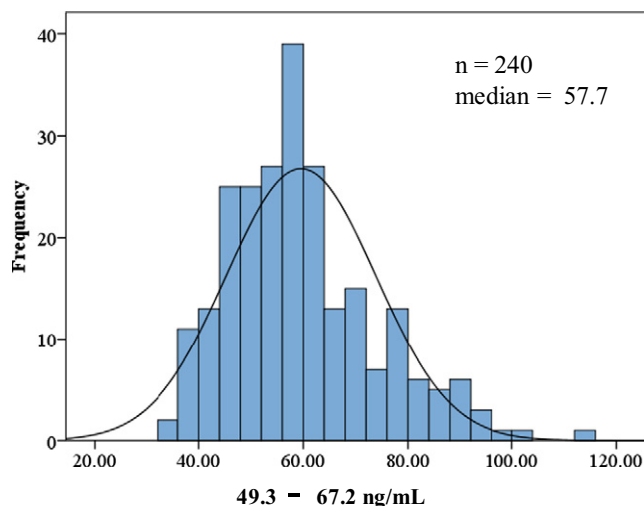

n = 240
median = 57.7

49.3 - 67.2 ng/mL

Fig. 6. Histogram of the LPL concentration in healthy volunteers (Japan) in the pre-heparin serum. There was no distribution difference of LPL concentration between males and females. The median and 25% tile to 75% tile (RI; reference interval) values of the LPL concentration are shown.

These results were in agreement with the results determined using ELISA [18].

3.6. Interference

Interference tests were performed by adding potentially interfering substances to serum pools and examining any changes in the absorbance values (Fig. 3). We investigated the effects of free bilirubin F and C, hemoglobin, triglyceride and rheumatoid factor on the measurement of LPL. The results showed that bilirubin F and C up to 200 mg/l did not affect the assay precision. Similarly, hemoglobin up to 5 g/l, triglyceride up to 1500 fonnazin turbidity units (FTU) and rheumatoid factor up to 500 IU/ml did not alter the assay precision. The recovery of LPL was within 10% of the original concentration (data not shown). These results suggest that the substances tested here do not interfere with the ability of the assay to measure LPL accurately.

3.7. Correlation of LTIA and ELISA

For comparison of the LTIA and LPL assays with the established ELISA assay (Sekisui Medical), blood samples were analyzed by two different range calibrators in both the pre-heparin serum and post-heparin plasma. As shown in Fig. 4A, regression statistics calculated in the tested sera (n = 40) gave y(LTIA)=0.845x(ELISA)+4.433, r = 0.965. Even in a relative high range in post-heparin plasma (Fig. 4B), using an LPL value between 0 and 600 ng/ml (n = 40), the correlation result was y(LTIA)= 0.871x(ELISA)+10.114, r = 0.942, indicating a good correlation between the two methods. A Bland–Altman plot was constructed to visualize the differences between the two methods [21]. The difference plot confirmed that there was a difference between the values measured with LTIA and ELISA, and the 95% confidence limits of the difference

Table 2
The univariate correlation coefficient analysis between the LPL concentration and the lipid parameters.

Analyte	r_s	p
Triglyceride	−0.237	0.0003
LDL cholesterol	0.0908	NS
HDL cholesterol	0.3659	<0.0001
RLP cholesterol	−0.1909	0.0036
Small, dense LDL cholesterol	0.0242	NS
Apolipoprotein C-III	0.0961	NS

were ± 13 ng/ml and ± 31 ng/ml, respectively. The mean difference between the two immunoassays was 6.8 ng/ml and 45 ng/ml at these levels, suggesting that there was no consistent bias between the two methods. A somewhat greater variance was seen at an LPL level >300 ng/ml. This was not considered to reduce the reliability or potential usefulness of the assay, since the potential interest regarding risk assessment lies in the low range.

3.8. Plasma LPL concentrations in healthy volunteers

The median pre-heparin serum LPL (Fig. 5A) and post-heparin plasma LPL (Fig. 5B) concentration in 40 healthy US volunteers in the fasting state were 50.3 ng/ml (range 50–77 ng/ml (25% tile to 75% tile)) and 381 ng/ml (range 354–411 ng/ml (25% tile to 75% tile)), respectively. The median pre-heparin LPL concentration in 240 healthy Japanese subjects (170 male, 70 female aged 20–72 years) in fasting state was 57.7 ng/ml (range 49–67 ng/ml (25% tile to 75% tile)) (Fig. 6).

No significant difference in the LPL concentration was detected between the serum and EDTA plasma specimens. Table 2 shows the univariate correlation coefficient analysis among the lipid parameters. LPL is significantly and positively correlated with HDL-C and inversely correlated with TG and RLP-C (Table 2).

4. Discussion

The main purpose for the development of the LPL latex assay system was to determine the pre-heparin serum LPL concentration for routine clinical laboratory use in a manner that does not require heparin injection before blood withdrawal. Heparin injection has been a great barrier for the determination of LPL in clinical practice. Although LPL in the pre-heparin serum is known to not have any activity, its concentration is high enough to be detected by a latex assay system as well as by ELISA. To the best of our knowledge, this is the first report of the determination of the serum LPL concentration with a latex assay system. This system provides a rapid and sensitive LPL assay for routine clinical practice and may be applied to large epidemiological studies using frozen samples.

In order to determine the pre-heparin LPL concentration in ordinary clinical laboratories, we have developed a latex particle-enhanced turbidimetric immunoassay (LTIA) for the measurement of LPL concentration over a wide range in both the pre-heparin serum and post-heparin plasma, and compared the result with an ELISA assay currently in use [18]. The assay presented here takes only 10 min on an automated analyzer. Intra-assay CVs of the ELISA were in the range of 2.2–5.5%. Therefore, the superior reproducibility of our results adds further value to this convenient assay system. Therefore, the monitoring of a low range of LPL, even less than 10 ng/ml, seems to be available for clinical use. The analytical detection limit of this assay was 10.7 ng/ml and is thus available for the detection of hyperlipidemic serum. This value is equal to the reported lower detection limit of ELISA. In respect to the precision and analysis time, the LTIA assay is superior to the ELISA assay and highly correlated with the automated LPL activity assay in post-heparin plasma reported by Imamura et al. (data not shown) [22].

The reproducibility, simplicity and full automation capacity with a widely used clinical chemistry analyzer like the Hitachi 7700 P are the key to routine clinical diagnosis. The LTIA assay offers the advantages of being precise, easy to perform and more rapid than the ELISA test as an adiponectin latex assay system [23].

The post-heparin LPL concentrations were 5 to 10 fold higher than pre-heparin LPL, but the pre-heparin LPL concentrations by LTIA were the levels within the range of accurate measurement. Also, in healthy Japanese subjects, the pre-heparin LPL concentration was inversely correlated with TG and RLP-C, and positively correlated with HDL-C, which are the same results shown by LPL-ELISA assay [18].

The measurement of the pre-heparin LPL concentration may prove to be clinically meaningful, because it is possible to identify LPL deficiency cases, such as type IV and V with a high TG concentration, without any need of a heparin injection.

In conclusion, this study has shown that the latex particle-enhanced turbidimetric immunoassay evaluated here is highly correlated with LPL-ELISA. It is a robust, easily implemented tool for measuring LPL concentrations in the clinical laboratory without the need of a heparin injection.

References

[1] Nilsson-Ehle P, Garfinkel AS, Schotz MC. Lipolytic enzymes and plasma lipoprotein metabolism. Annu Rev Biochem 1980;49:667–93.
[2] Brunzell JD, Deeb SS. Familial lipoprotein lipase deficiency, apo C-II deficiency and hepatic lipase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular basis of inherited disease. New York: Mc Graw-Hill Inc.; 2001. p. 2789–816.
[3] Beisiegel U, Weber W, Bengtason-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. Proc Natl Acad Sci U S A 1991;88:8342–6.
[4] Chappell DA, Fry GL, Waknitz MA, Iverius P-H, Williams SE, Strickland DK. The low density lipoprotein receptor-related protein/a2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. J Biol Chem 1992;267:25764–7.
[5] Eisenberg S, Sehayek E, Olivecrona T, Vlodavsky I. Lipoprotein lipase enhances binding of lipoproteins to heparan sulphate on cell surfaces and extracellular matrix. J Clin Invest 1992;90:2013–21.
[6] Nylcjaer A, Bengtason-Olivecrona G, Lookene A, et al. The a2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and a-migrating very low density lipoprotein associated with the lipase. J Biol Chem 1993;268:15048–55.
[7] Peterson J, Fujimoto WY, Brunzell JD. Human lipoprotein lipase: relationship of activity, heparin affinity, and conformation as studied with monoclonal antibodies. J Lipid Res 1992;33:1165–70.
[8] Ikeda Y, Takagi A, Ohkaru Y, et al. A sandwich-enzyme immunoassay for the quantification of lipoprotein lipase and hepatic triglyceride lipase in human postheparin plasma using monoclonal antibodies to the corresponding enzymes. J Lipid Res 1990;31:1911–24.
[9] Endo K, Miyashita Y, Saiki A, et al. Atorvastatin and pravastatin elevated pre-heparin lipoprotein lipase mass of type 2 diabetes with hypercholesterolemia. J Atheroscler Thromb 2004;11:341–7.
[10] Kinoshita T, Shirai K, Itoh M. The level of pre-heparin serum lipoprotein lipase mass at different stages of pregnancy. Clin Chim Acta 2003;337:153–6.
[11] Kobayashi J, Saito K, Fukamachi I, et al. Pre-heparin plasma lipoprotein lipase mass: its correlation with intra-abdominal visceral fat accumulation. Horm Metab Res 2001;33:412–6.
[12] Miyashita Y, Shirai K, Itoh Y, et al. Low lipoprotein lipase mass in preheparin serum of type 2 diabetes mellitus patients and its recovery with insulin therapy. Diabetes Res Clin Pract 2002;56:181–7.
[13] Totsuka M, Miyashita Y, Ito Y, et al. Enhancement of preheparin serum lipoprotein lipase mass by bezafibrate administration. Atherosclerosis 2000;153:175–9.
[14] Hitsumoto T, Ohsawa H, Uchi T, et al. Preheparin serum lipoprotein lipase mass is negatively related to coronary atherosclerosis. Atherosclerosis 2000;153:391–6.
[15] Hitsumoto T, Yoshinaga K, Aoyagi K, et al. Association between preheparin serum lipoprotein lipase mass and acute myocardial infarction in Japanese men. J Atheroscler Thromb 2002;9:163–9.
[16] Saiki A, Oyama T, Endo K, et al. Preheparin serum lipoprotein lipase mass might be a biomarker of metabolic syndrome. Diabetes Res Clin Pract 76;76:93–101.
[17] Shirakawa T, Nakajima K, Shimomura Y, et al. Comparison of the effect of post-heparin and pre-heparin lipoprotein lipase and hepatic triglyceride lipase on remnant lipoprotein metabolism. Clin Chim Acta 2014;440C:193–200.
[18] Kobayashi J, Hashimoto H, Fukamachi I, et al. Lipoprotein lipase mass and activity in severe hypertriglyceridemia. Clin Chim Acta 1993;216:113–23.
[19] Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322–34.
[20] Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. Int J Obes (Lond) 2014;38:812–7.
[21] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
[22] Imamura S, Kobayashi J, Nakajima K, et al. A novel method for measuring human lipoprotein lipase and hepatic lipase activities in postheparin plasma. J Lipid Res 2008;49:1431–7.
[23] Nishimura A, Sawai T. Determination of adiponectin in serum using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer. Clin Chim Acta 2006;371:163–8.