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Quantitation of Plasma Apolipoprotein A-I with a Sandwich Type Enzyme-Linked Immunosorbent Assay Using Monoclonal Antibodies

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Abstract: A sandwich-type enzyme-linked immunosorbent assay (ELISA) for the quantification of human apolipoprotein A-I (apoA-I) was developed using monoclonal antibodies. For this assay, we used three monoclonal antibodies to trap and detect apo A-I. HDAI16 and HDAI5 monoclonal antibodies were used for trapping apoA-I and HDAI8 monoclonal antibody was for detecting apoA-I. These three monoclonal antibodies were produced by immunizing mice with high density lipoprotein (HDL) isolated from human plasma. By immunoblot analysis, these three monoclonal antibodies were specific to apoA-I and showed no cross-reactivities with other plasma proteins. The results of competition assays for epitope cross-reactivity test also verified that these monoclonal antibodies identified separate and distinct epitopes on HDL and apoA-I. Affinity constants of monoclonal antibodies were measured by ELISA. Their association constants ranged from 10^7 to 10^8 M^{-1}. For this assay, pure apoA-I was isolated by affinity chromatography using monoclonal antibodies. In this sandwich assay, the amount of HRP-labeled HDAI8 bound to apoA-I trapped by HDAI16 and HDAI5 was proportional to apoA-I concentration in the range of 0 to 500ng/ml. ApoA-I concentration in plasma was calculated from the linear regression equation of standard curve. The precision and reliability of the assays are reflected in the low intra- and interassay coefficients of variation that averaged 3.25% and 4.30%, respectively. This assay is sensitive, simple, reproducible, convenient in incubation interval, and does not use radioisotope; thus it can be widely applied in clinical laboratories.

Key words: affinity constant, apolipoprotein A-I, enzyme-linked immunosorbent assay, high density lipoprotein, monoclonal antibody

Apolipoprotein A-I (apoA-I), a major protein component of high density lipoprotein (HDL), plays an important role in lipoprotein metabolism and lipid transport. It stabilizes HDL particle structure and acts as an activator of lecithin:cholesterol acyltransferase (LCAT) (Eisenberg, 1984). Human apoA-I is a single polypeptide chain composed of 243 amino acids lacking cysteine. The level of HDL is inversely related to the risk of developing coronary artery disease (Gordon *et al.*, 1977; Wilson *et al.*, 1988). This inverse relationship is true both for morbidity of and mortality from coronary disease. The prognosis of recovery from myocardial infarction is also associated with plasma HDL level (Goldbourt *et al.*, 1986). The two most commonly used methods to measure HDL are to determine its lipid content in the form of cholesterol or its apoA-I content. It has been suggested that apoA-I level in human plasma is a better risk predictor than HDL cholesterol (Maciejko *et al.*, 1983). A convenient and accurate method for the determination of plasma apoA-I level should be helpful for the diagnosis and treatment of patients with coronary artery disease. To measure apoA-I value in plasma, a number of immunoassay methods, such as immunoturbidimetry (Dona *et al.*, 1987: Brustolin *et al.*, 1991), immunonephelometry (Maciejko *et al.*, 1987) and enzyme immunoassay (Betard *et al.*, 1987; Hogle *et al.*, 1988), have been reported. In spite of availability and sensitivity of ELISA, a standardized assay method for the measurement of apoA-I using ELISA is not available yet because of substantial variation among laboratories. In addition, international standardization for measurements of apoA-I have been performed for radial immunodiffusion, nephelometry, and turbidimetry, but not

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ELISA (Marcovina *et al.*, 1993). The reasons for these discrepancies are diverse (Marcovina & Albers, 1989).

Here we report the development of a sandwich-type ELISA for the quantification of apoA-I using two monoclonal antibodies (MAbs) for trapping and a MAb to detecting apoA-I. The reproducibility and reliability of the assays are reflected in the low intra- and interassay coefficients of variation (CVs) that averaged 3.25% and 4.30%, respectively. It is expected that this ELISA assay with low CVs can overcome substantial variation among laboratories.

Materials and Methods

Materials

CNBr-activated Sepharose 4B beads were purchased from Pharmacia Biotech. (Uppsala, Sweden). Horseradish peroxidase (HRP), pristane, and mouse MAb isotyping reagents, phenylmethylsulfonyl fluoride, aprotinin, O-phenylenediamine (OPD), BSA, and H_2O_2 were from Sigma Chemical Co. (St. Louis, USA); HRP-conjugated goat anti-mouse IgG antibody from Calbiochem; Balb/c mice for monoclonal antibody production, from Myung Jin Instrument Co. (Seoul, Korea); 96-well polystyrene microtiter plate from Corning Co. (Corning, USA).

Purification of plasma lipoproteins

Fresh frozen plasma from human, obtained from Korea Red Cross (Seoul, Korea) was thawed. one mM EDTA, 0.005% gentamycin, 0.015% phenylmethyl sulfonyl fluoride, and 10 U/ml aprotinin were immediately added to the plasma (Edelstein *et al.*, 1986). To remove chylomicrons, the plasma was ultracentrifuged for 30 min at 4°C at 35,000 rpm with SW41 Beckman rotor. From plasma without chylomicron, total lipoprotein fraction was isolated by ultracentrifugation at d=1.221. Using Bio-Gel A-5m (6%(w/v) agarose) chromatography, three distinct fractions, i.e. VLDL, LDL and HDL were preparatively separated from total lipoproteins (Rudel *et al.*, 1974) and visualized by SDS-PAGE.

Preparation of monoclonal antibodies

Each BALB/c mouse was immunized intraperitoneally with 25 μg of purified HDL in complete Freund's adjuvant. Subsequent injections were performed with incomplete Freund's adjuvant at three week intervals and antibody titer in mouse blood was measured by ELISA. Three days after the final intravenous injection of HDL, spleen cells were prepared by splenectomy. Spleen cells were fused with myeloma cell SP2/0 Ag-14 using PEG4000 (Galfre *et al.*, 1977). Hybridoma cells were selected in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum and hypoxanthine-aminopterine-thymidine complement. The culture supernatants were tested by immunoblotting and ELISA for their abilities to secrete specific antibodies against apoA-I. Positive hybridomas were cloned by limiting dilution method in Dulbecco's modified Eagle's medium containing hypoxanthine-aminopterine-thymidine complement. After the third cloning, the isotype of each antibody was determined by mouse monoclonal sub-isotyping kit. Hybridoma cells were injected into the peritoneum of BALB/c mice and ascites fluid containing the monoclonal antibody was obtained and used to determine the specificities of the antibodies against apoA-I by ELISA and immunoblotting. The monoclonal antibodies were further purified with a protein G affinity column (HiTrap, Pharmacia Biotech., Uppsala, Sweden) according to the manufacturer's instruction manual. The purity of antibodies was monitored by SDS-PAGE and their activities were measured by ELISA.

Immunoblotting

Five microliters of normal human plasma, 10 μg of VLDL, 10 μg of LDL, and 10 μg of HDL were boiled for 5 min in electrophoresis sample buffer containing 1% SDS, loaded onto a 5-20% acrylamide gradient slab gel containing SDS, and electrophoresed at 30 mA of constant current. Proteins were electrophoretically transferred to nitrocellulose membrane at 500 mA of constant current. Nitrocellulose membrane was blocked with TBS (20 mM Tris, 0.2 M NaCl, pH7.5) containing 1% BSA for 1 h at 37°C, and incubated with TBS-1% BSA containing 1 μg/ml monoclonal antibody for 1 h at 37°C. After washing with TBS containing 0.05% Tween 80, the antibody bound was detected by incubating with HRP-conjugated goat anti-mouse IgG for 1 h at 37°C. After washing, the band was visualized and exposed to X-ray film.

Preparation of apoA-I by affinity chromatography

Human HDL (d 1.603-1.210 g/ml) was obtained from human plasma by sequential ultracentrifugation (Schumaker & Puppione, 1986). After removing chylomicron, plasma was adjusted to d 1.063 g/ml with solid KBr and followed by ultracentrifugation at 35,000 rpm for 20 h at 4°C. The infranate was collected, adjusted to d 1.210 g/ml with solid KBr, overlayed with d=1.210 g/ml KBr solution and ultracentrifuged as above. Top fraction was obtained and delipidated by ethanol-diethylether solution (3:2, v/v). Coupling the purified MAbs to CNBr-activated Sepharose 4B was performed (Jang, *et al.*, 1994). The delipidated HDL dissolved in PBS buffer was applied on affinity gel. After then, the gel was washed away with excess PBS buffer and the bound ligand was eluted with PBS buffer containing 8 M urea. The

eluted fractions were collected and dialyzed against excess 0.01 M NH_4HCO_3. Their purities and activities were tested by SDS-PAGE. The purified apoA-I was quantified by modified Lowry-SDS method (Henderson, *et al.*, 1990).

Preparation of HRP-labeled MAbs

Highly purified MAbs were conjugated to HRP (type VI-A) as described by the modified periodate-mediated conjugation (Tijssen and Kurstak, 1984). HRP-IgG conjugates were separated from unconjugated IgG and free HRP components by applying onto a Sephadex G200 gel filtration chromatography. The HRP-conjugated MAb peak was identified by the RZ value (A_{403}/A_{280}) of about 0.3-0.5. The activity of HRP conjugate was determined (Catty, 1989).

Competition assay for epitope cross-reactivity test of MAbs

A 96-well microtiter plate was coated for 16 h at 4°C with 100 μl of 0.1 M carbonate buffer (pH 9.6) containing 2.5 μg/ml apoA-I or HDL. Various concentrations (0.312 μg/ml to 50 μg/ml) of MAbs were mixed with 20 ng of HRP-conjugated MAb. Each mixture was transferred into the wells of the plate and incubated for 1 h at 37°C. After washing three times with PBS buffer containing 0.05% Tween (PBST), 100 μl of freshly prepared substrate solution (50 mM citrate, 0.1 M Na_2HPO_4, 0.08% OPD, 0.012% H_2O_2, pH 5.0) was added and incubated for 20 min at room temperature. The reaction was stopped by addition of 50 μl of 2 M H_2SO_4 and the color of the solution was determined at 490 nm by an automatic 96-well plate reader (THERMOmax™, Molecular Devices Co., Meno Park, USA).

Measurement of affinity constant by ELISA

The apoA-I at various concentrations (3.125×10^{-9} to 100×10^{-9} M) was mixed with a constant amount of MAb (2×10^{-11} M) in PBS containing 10 mg/ml BSA. After overnight incubation at room temperature, each mixture was transferred and incubated for 1 h at room temperature into the wells of a microtiter plate previously coated with 2.5 μg/ml apoA-I. After washing with PBST, the bound MAbs were detected by adding HRP-conjugated goat anti-mouse IgG. Dissociation constant (Kd) and association constant (Ka) values were deduced from the slope calculated from linear regression (Friguet *et al.*, 1985: Jang *et al.*, 1996).

Sandwich ELISA

A 96-well microtiter plate was coated for 16 h at 4°C with 100 μl of 0.1 M carbonate buffer (pH 9.6) containing 2.5 μg/ml of HDAI16 and HDAI5. Residual binding sites on the plate were blocked by incubating with 200 μl PBS buffer containig 1% BSA for 1 h at 37°C. After washing three times with PBST, the plate coated with those monoclonal antibodies was dried for 20 min at 37°C in convection oven. The purified apoA-I was used as standard material. For standard curve, which was prepared for each plate, the apoA-I was diluted in PBS containig 1% BSA to provide apoA-I concentrations ranging form 0 to 500 ng/ml. Plasma samples were diluted 5000 fold in PBST containig 1% BSA. One hundred microliters of standards and unknown samples were pipetted into wells in triplicate. After incubating for 1 h at 37°C, the plate was washed four times with PBST and 100 μl of HRP-conjugated HDAI 8 MAb diluted 5000 fold in PBS containig 1% BSA was added. After incubating for 1 h at 37°C and subsequent washing, 100 μl of freshly prepared substrate solution was added and incubated for 20 min at room temperature. The reaction was stopped by adding 50 μl of 2 M H_2SO_4 and the color of the solution was determined at 490 nm.

Results

In this sandwich type ELISA, immobilized monoclonal antibodies HDAI5 and HDAI16 were used as the capture antibodies and soluble antibody HDAI8 conjugated with horseradish peroxidase was used to detect the bound apoA-I. HDAI5, HDAI8 and HDAI16 monocloal antibodies were generated with fresh HDL as immunogen. These monoclonal antibodies also bound to both isolated apoA-I and native HDL. For this solid phase assay to be successful, these MAbs must specifically bind to apoA-I. The specific ability of these three antibodies to bind apoA-I was tested by Western blot technique. Initially, total lipoprotein fraction was isolated by ultracentrifugation at d=1.221 and distinct fractions, i.e. VLDL, LDL, HDL were preparatively separated from total lipoproteins using Bio-Gel A-5m (6% (w/v) agarose) chromatrography (Fig. 1A), and then immunoblotting was performed. HDAI16 bound to only apoA-I present in HDL and normal human plasma, but did not bind to any other proteins present in lipoproteins and normal human plasma (Fig. 1B). Immunoblotting of HDAI5 and HDAI8 also showed similar results (data not shown). These results indicate that these antibodies are specific to apoA-I. To use apoA-I in various assays, HDL was first purified by sequential ultracentrifugation. ApoA-I was isolated from ether/ethanol-delipidated HDL by affinity chromatography using our monoclonal antibodies. Purity of the purified apoA-I was verified by SDS-PAGE analysis (Fig. 2).

Next, verification that HDAI5, HDAI8 and HDAI16

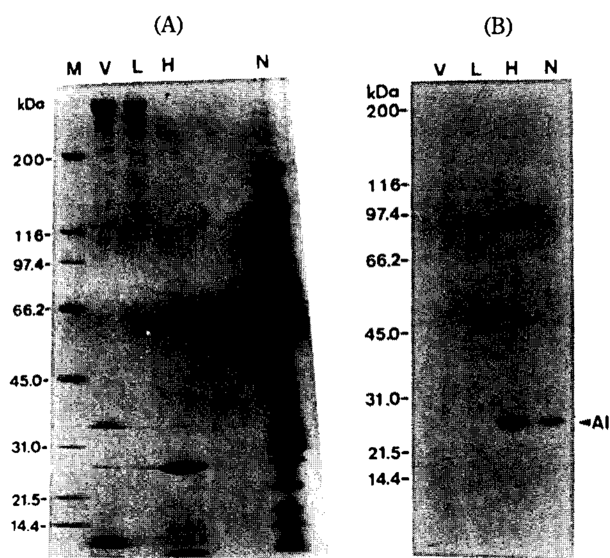

Fig. 1. Characterization of binding specificity of HDAI16 MAb by immunoblotting analysis. A 5-20% acrylamide gradient slab gel loaded with 10 μg of VLDL (lane V), 10 μg of LDL (lane L), 10 μg of HDL (lane H) and 5 μl of normal human plasma (lane N) was electrophoresed at 30 mA of constant current (A), transferred to nitrocellulose membrane, blocked with BSA, and incubated with monoclonal antibody. The bound antibody was detected by HRP-conjugated goat anti-mouse IgG, visualized, and exposed on X-ray film (B).

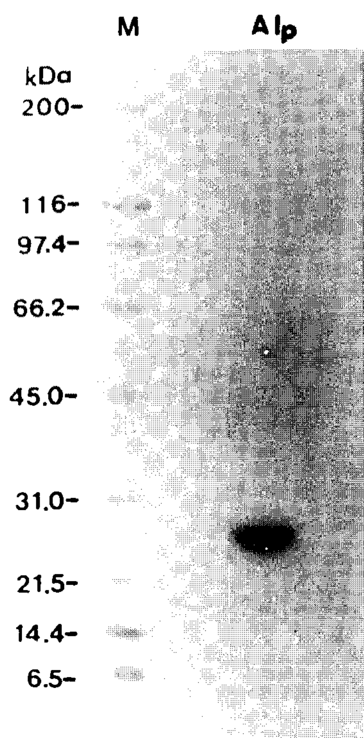

Fig. 2. SDS-PAGE of apoA-I isolated by affinity chromatography. An 5-20% acrylamide gradient slab gel loaded with 10 μg of purified apoA-I (lane AIp) was electrophoresed at 30 mA of constant current. The purified apoA-I migrated to a molecular weight of 28,000 as single band on SDS-PAGE.

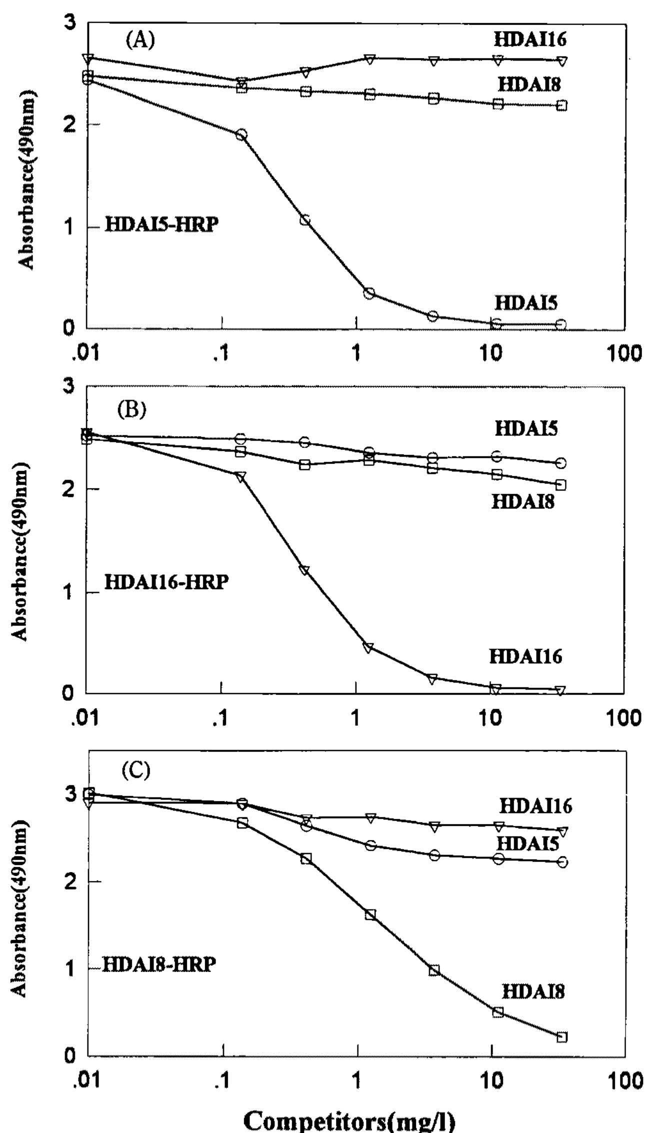

Fig. 3. Competition of HRP-labeled HDAI16, HDAI5 and HDAI8 with unlabeled HDAI16, HDAI5 and HDAI8 for binding to apoA-I. HRP-labeled HDAI15 (A), HDAI6 (B) and HDAI8 (C) were competed with increasing concentrations of unlabeled HDAI16, HDAI5 and HDAI8 in 96-well microtiter plate coated with HDL. After washing, HRP-labeled goat anti-mouse IgG was incubated to detect apoA-I and color was developed.

antibodies identified separate and distinct epitopes on HDL was obtained by competition assay. A 125-fold excess of purified antibodies HDAI8 and HDAI16 did not compete wih a limiting amount of HRP-labeled HDAI5 for binding to the purified HDL (Fig. 3A). Similarly, a 125-fold excess of other unlabeled antibodies did not compete with a limiting amount of HRP-labeled antibody for binding to HDL (Fig. 3B & 3C). Similar results were obtained with apoAI-coated plate (data not shown). These results indicate that these monoclonal antibodies recognize separate and distinct epitopes on HDL and apoA-I.

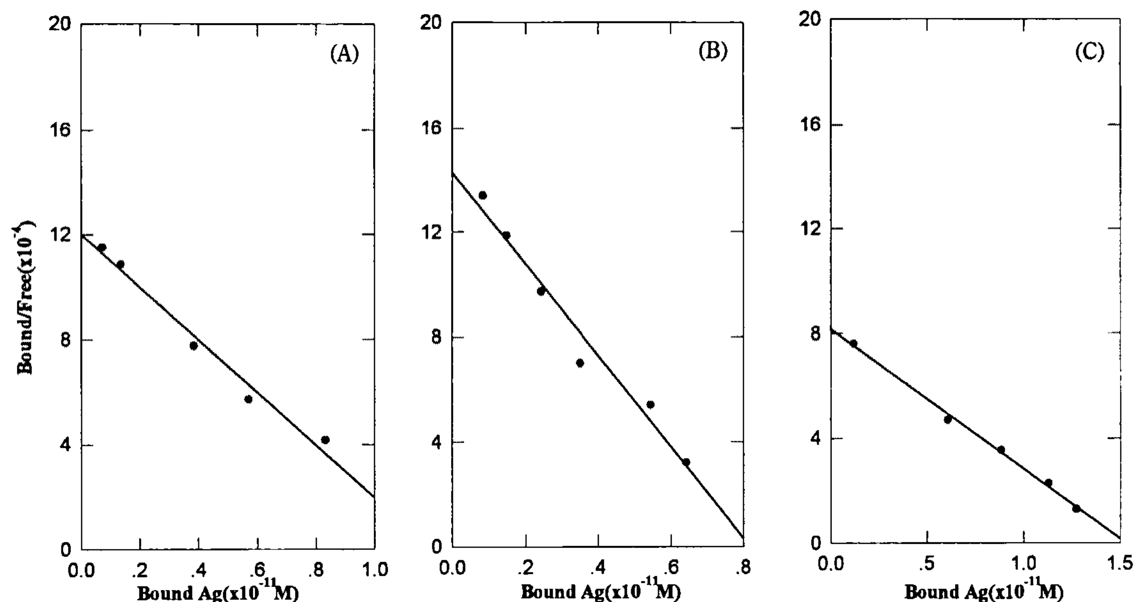

Fig. 4. Scatchard plots of the binding of HDAI16 (A), HDAI5 (B) and HDAI8 (C) to apoA-I measured by ELISA. A 96-well microtiter plate coated with apoA-I was incubated with serially diluted apoA-I mixed with HDAI16 (A), HDAI5 (B) and HDAI8 (C). Ka and Kd values were deduced from the slopes calculated from linear regression.

Table 1. Characteristics of monoclonal antibodies against ApoA-I

	HDAI16	HDAI5	HDAI8
Reactants	ApoA-I, HDL	ApoA-I, HDL	ApoA-I, HDL
Isotype	IgG1	IgG1	IgG2b
Ka[a]	1.11×10^{8} M^{-1}	1.74×10^{8} M^{-1}	5.37×10^{7} M^{-1}
Kd[b]	9.01×10^{-9} M	5.75×10^{-9} M	1.86×10^{-8} M

[a]Ka: Association constant
[b]Kd: Dissociation constant

Affinity constants of HDAI5, HDAI8 and HDAI16 were determined by ELISA using apoAI-coated plate (Fig. 4). The association constants that were deduced from the slopes calculated from linear regression of Scatchard plots are as follows: 1.11×10^{8} M^{-1} for HDAI16, 1.74×10^{8} M^{-1} for HDAI5 and 5.37×10^{7} M^{-1} for HDAI8 (Table 1).

Binding studies that used HDAI5 and HDAI16 as the capture antibodies and HRP-labeled HDAI8 as detection antibody to quantitate apoA-I were performed. Increasing concentrations of HDL and apoAI were added to make a standard curve. To calibrate our in-house calibrator, three fresh-frozen serum pools, Blue8, Green8 and Yellow 8, were purchased from Northwest Lipid Research Laboratory (NWLRL) (Marcovina *et al.*, 1993; Marcovina *et al.*, 1994). ApoA-I values of Blue8, Green8, and Yellow8 were 138±5, 117±5, and 163±3 mg/ml, respectively. The target values from these serum pools were transfered to our in-house standards. After calibration, the results were plotted on the basis of apoA-I (Fig. 5). The correlation coefficient (r) of the curve was 0.998. ApoA-I value in serum that was able to be deduced from the linear regression equation of the standard curve of apoAI was calculated by the following equation: ApoA-I value in serum (g/L)=1.572×absorbance at 490 nm−0.13. As determined by slope analysis, HDL and apoA-I were recognized with similar affinities in this assay.

Table 2. Intra- and interassay variability of the sandwich assay of ApoA-I

A) Intraassay			
Specimens	Serum 1	Serum 2	Serum 3
Replicates	10	10	10
Mean (mg/dl)	175.6	160.8	126.1
S.D. (mg/dl)	6.10	4.11	5.00
CV (%)	3.22	2.55	3.97
B) Interassay			
Specimens	Serum A	Serum B	Serum C
Replicates	12	12	12
Mean (mg/dl)	112.8	151.4	175.7
S.D.[a] (mg/dl)	4.28	7.26	7.56
CV[b] (%)	3.79	4.79	4.30

[a]S.D: standard deviation
[b]CV: coefficient of variation (S.D./mean)

To validate our assay for apoA-I, intra- and interassay were performed. The intraassay CVs, which were determined by assaying three different plasma samples 10 times in a single assay, ranged from 2.55% to 3.97% (Table 2A). The interassay CVs, which were determined by assaying three different plasma samples 12 times, ranged from 3.79% to 4.79% (Table 2B). To assess the accuracy of the assay, we performed recovery experi-

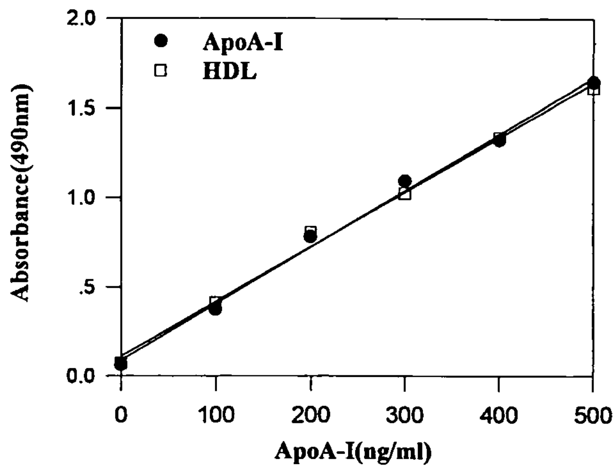

Fig. 5. Representative standard curve of the sandwich ELISA. One hundred microliters of apoA-I and HDL of 0 to 500 ng/ml on the basis of apoA-I content was applied to each well coated with 2.5 μg/ml of HDAI16 and HDAI5, and incubated for 1 h at 37°C. After washing, HRP-labeled HDAI8 was incubated to detect apoA-I and color was developed.

Table 3. Accuracy of the sandwich assay of ApoA-I

A) Recovery of ApoA-I added to endogenous serum

	$ApoAI_{endogenous}$ (mg/dl)	$ApoAI_{added}$ (mg/dl)	$ApoAI_{observed}$ (mg/dl)	Recovery (%)
Serum X	89.0	40	127.8	97.0
		80	169.0	100.0
Serum Y	100.2	40	145.7	113.8
		80	183.2	103.8
Serum Z	149.7	40	190.5	102.0
		80	230.9	101.5

B) ApoA-I concentrations determined at different dilutions of serum

Dilution	Serum 4 (mg/dl)	Serum 5 (mg/dl)	Serum 6 (mg/dl)
1 : 5,000	97.2	110.0	185.4
1 : 10,000	99.4	114.0	187.4
1 : 20,000	102.4	115.6	190.0
Mean	99.7	113.2	187.6
S.D.	2.63	2.90	2.31
CV (%)	2.64	2.56	1.23

ments in which known quantities of isolated apoA-I standard were added to normal plasma samples, which were then assayed as unknowns. In these experiments the observed recovery of apoA-I ranged from 97% to 113% (Table 3A). No significant difference was found in total apoA-I concentration in plasma when a fourfold range of dilutions of plasma was assayed (Table 3B).

Dicussion

In this report, we developed a sandwich-type ELISA that used HDAI5 and HDAI16 for the capture antibodies and HRP-labeled HDAI8 for detective antibody to quantify apoA-I in plasma. These three monocloal antibodies were generated with fresh HDL instead of apoA-I as immunogen because antibodies generated with fresh HDL bound to heterogeneous HDL more efficiently than antibodies generated with purified apoA-I (Curtiss and Edgington, 1985). Furthermore, because of possible conformational change of apoA-I structure after isolation, MAbs generated with isolated apoA-I might also have less or no binding affinity with the native form of apoA-I than antibodies generated with fresh HDL. These monoclonal antibodies, HDAI5, HDAI8 and HDAI16 were able to react with both HDL and apoA-I. They were specific to apoA-I because they did not cross-react with VLDL, LDL, various apolipoproteins or other proteins in plasma when immunoblotted (Fig. 1). To use apoA-I as in-house calibrators and in various assay, apoA-I was highly purified from ether/ethanol-delipidated HDL by affinity chromatography using monoclonal antibodies. Competition results described here confirm that these three antibodies recognized distinct epitopes on apoA-I, indicating that they would be ideally suitable for use in a sandwich-type immunoassay. Affinity constants of monoclonal antibodies were measured by ELISA. Their association constant(Ka)s ranged from 10^7 to 10^8 M^{-1}.

In our assay, the mixture of two MAbs, HDAI5 and HDAI16 with relatively high affinity constants was used for effective capture of apoA-I in plasma. The result of this assay showed that the amount of HRP-labeled HDAI8 bound to apoA-I trapped by capture antibodies was proportional to apoA-I concentration. Plasma apoA-I concentration was calculated by deducing from linear regression equation of apoA-I standard curve. The intraassay CVs ranged from 2.55% to 3.97% and the interassay CVs ranged from 3.79% to 4.79% showed good reproducibility, reliability and precision of this assay. The observed recovery of apoA-I of 97% to 113% and no significant difference in total apoA-I concentration within a fourfold range of dilutions of plasma also showed high accuracy and precision of this assay.

The sandwich type ELISA to quantitate apoA-I in plasma offers distinctive advantages over radial immunodiffusion, electroimmunoassay, immunonephelometry, immunoturbidimetry and radioimmunoassay. It is not affected by sample turbidity, no radioisotopes are used, and monoclonal antibodies, which are highly specific and available in large quantities are easily purified more than polyclonal antibody. Also, this assay is simple and can be completed within 3 hours. The precision and reliability of the assay are reflected in low intra- and interassay CVs that averaged 3.25% and 4.30%, respectively. This result might have advantages over other EL-

ISA. It is also expected that this ELISA assay with low CVs can overcome substantial variation of ELISA among laboratories. Further, because we also developed a sandwich type ELISA to quantitate apoB (Jeong *et al.*, 1995), we are considering to commercialize this ELISA assay and participate in international standardization for the measurements of apoA-I and apoB.

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References

Antonio, M. G. Jr., Pownall, H. J. and Havel, R. J. (1986) *Methods Enzymol.* **128**, 3.

Betard, C., Vu-Dac, N. and Mezdour, H. (1987) *J. Clin. Chem. Clin. Biochem.* **25**, 892.

Brustolin, D., Maierna, M., Aguzzi, Zoppi, F., Tarenghi, G. and Berti, G. (1991) *Clin. Chem.* **37**, 742.

Catty, D. (1989) Antibodies (Vol. II): A Practical Approach, IRL Press at Oxford University Press, Oxford.

Curtiss, L. K. and Edgington, T. S. (1985) *J. Biol. Chem.* **260**, 2982.

Dona, V., Papagni, M., Tarenghi, G., Aguzzi, F. and Berti, G (1987) *Giorn. It. Chim. Clin.* **12**, 205.

Edelstein, C. and Scanu, A. M. (1986) *Methods Enzymol.* **128**, 151.

Eisenberg, S. (1984) *J. Lipid. Res.* **25**, 1017.

Friguet, B., Chaffotte, A. F., Djavadi-Ohaniance, L. and Goldberg, M. E. (1985) *J. Immunol. Methods* **77**, 305.

Galfre, G. and Milstein, C. (1981) *Methods Enzymol.* **73**, 3.

Goldbourt, U., Cohem, L. and Neufeld, H. N. (1986) *Int. J. Epidemiol.* **15**, 51.

Gordon, T., Castelli, W. T., Hjortland, M. C., Kannel, W. B. and Dawber T. R. (1997) *Am. J. Med.* **62**, 707.

Henderson, L. O., Powell, M. K., Smith, S. J. Hannon, W. H., Cooper, G. R. and Marcovina, S. M. (1990) *Clin. Chem.* **36**, 1911.

Hogle, D. M. Smith, R. S. and Curtiss, L. K. (1988) *J. Lipid. Res.* **29**, 1221.

Jang, J. S., Kim, J. M., Chung, G. H., Paik, B. H. and Kim, H. J. (1996) *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **29**, 192.

Jang, S-Y., Cheong, T-K., Shim, W, Kim, J-W. and Park, K-H. (1994) *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **27**, 38.

Jeong, J-Y., Kim, H-J., and Jue, D-Y. (1995) *Korean J. Biochem.* **27**, 81.

Maciejko, J. J., Holmes, D. R., Kottke, B. A., Zinsmeister, A. R., Dinh, D. M. and Mao, S. J. T. (1983) *N. Engl. J. Med.* **309**, 385.

Maciejko, J. J., Levinson, S. S., Markyvech, L., Smith, M. F. and Blevins, R. (1987) *Clin. Chem.* **33**, 2065.

Marcovina, S. M. and Albers, J. J. (1989) *Clin. Chem.* **35**, 2009.

Marcovina, S. M., Albers, J. J., Henderson, L. O. and Hannon, W. H (1993) *Clin. Chem.* **39**, 773.

Marcovina, S. M., Albers, J. J., Kenndy, H., Mei, J. V. Henderson, L. O. and Hannon, W. H (1994) *Clin. Chem.* **40**, 786.

Rudel, L. L., Lee, J. A., Morris, M. D. and Felts, J. M. (1974) *Biochem. J.* **139**, 89.

Schumaker, V. N. and Puppione, O. L. (1986) *Methods Enzymol.* **128**, 155.

Tijssen, P. and Kurstak, E. (1984) *Anal. Biochem.* **136**, 451.

Wilson, P. W. F., Abbott, R. D. and Castelli, W. P. (1988) *Arteriosclerosis* **8**, 737.