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A rapid, simple measurement of human albumin in whole blood using a fluorescence immunoassay (I)

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Abstract

Background: Human serum albumin (HSA) is the most abundant plasma protein and plays key a role in metabolism. The variation in albumin concentration provides valuable information related to metabolic diseases and diagnostic application. *Methods:* We constructed two assay systems to quantify the albumin concentration. The immunoassay used a fluorescence (FL) dye to detect albumin in samples and employed the conventional chromatography as a separation system. The assay system consists of an anti-HSA-mAb or an HSA immobilized test strip in a disposable cartridge, a fluorescence-labeled detector buffer and a laser-fluorescence scanner. We mixed the sample with detector, loaded it onto a cartridge, incubated it for 10 min and measured the concentration of albumin in a laser-fluorescence scanner. We examined the comparability of assay with an automated BCG dye binding method using a Hitachi 747 biochemical analyzer. *Results:* The correlation of coefficient between A_T/A_C as converted from the relative fluorescence units (RFU) and albumin concentration displayed reasonable reliability in both the competition and the inhibition assay systems ($r = 0.998$). Using the Bland–Altman difference plot analysis, we observed an acceptable agreement between two methods, the fluorescence immunochromatography assay (FL-ICA) and the automated BCG dye-binding method of a Hitachi biochemical analyzer, over the clinical relevant range of HSA concentrations. The coefficient of variation (CV) of within- and between-run variation in the immunoassay system was $< 8\%$ and the recovery fell within 5% in each control sample. In addition to its reliable analytical performance, the assay with whole blood can be completed in 12 min using a one-step operation without any pretreatment. *Conclusion:* The developed immunoassay system using fluorescence dye and lateral-flow chromatography is a simple, fast and reliable method for quantifying the albumin concentration in whole blood.

Keywords: Fluorescence immunochromatography assay; Laser-fluorescence scanner; BCG dye-binding method; Human serum albumin

Abbreviations: HSA, human serum albumin; mAb, monoclonal antibody; FL-ICA, fluorescence immunochromatography assay; anti-HSA-FL, fluorescence-labeled anti-HSA monoclonal antibody; HSA-FL, fluorescence-labeled human serum albumin; anti-RIgG-FL, fluorescence-labeled anti-rabbit IgG; RFU, relative fluorescence units; CV, coefficient of variation.

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1. Introduction

Human serum albumin (HSA) is the most abundant plasma protein synthesized exclusively in the liver. HSA constitutes over half of the total plasma proteins, a concentration of 35–50 g/l, in a healthy individual [1]. Albumin plays important roles in the human body. One of its main functions is to regulate plasma osmotic pressure between the blood and tissues. This function is attributable to its high plasma concentration and relatively low molecular weight. One very important role of albumin is that it also functions as a transport molecule. This role is based on albumin's unique ability to bind a variety of exo- and endogenous compounds [2]. Thus, albumin can serve as a circulating depot of metabolites and as an extracellular antioxidant [3]. In addition, some research suggests that albumin may be a source of amino acids for various tissues [1]. Variation in human albumin concentration provides valuable information related to metabolic diseases and has various diagnostic applications. For example, a low concentration of serum albumin has been found in patients with certain hepatic diseases, inflammation, extensive burns and malnutrition [1,4]. A high concentration of serum albumin is usually seen in dehydrated patients. Urine albumin also has been used as an indicator of renal diseases including nephritic syndrome and uremia [5]. Specially, microalbuminuria, a urinary albumin excretion between 20 and 300 mg/l, is an accepted predictive marker for the early detection of renal disease and plays a key role in the identifying patients at high risk of developing complications of cardiovascular diseases and diabetes [6–8].

The albumin concentration can be measured using electrophoretic, dye-binding and immunological methods. For the past 30 years, researchers have based most of their measurements of serum albumin on two dye-binding methods, bromcresol green (BCG) and bromcresol purple (BCP) [9]. Some researchers question the lack of specificity of dye-binding assays for albumin with serum samples [10,11]. However, dye-binding assay for albumin with serum samples have been used for a long time because they are simple, sensitive and inexpensive [2]. As far as specificity is concerned, the immunological method is perhaps a good choice among the analytical assays. To measure HSA immunologically, one must dilute the serum samples because there is such a high concentration of albumin in the blood. Immunoassays for HSA are regarded as more appropriate for other body fluids (e.g. urine and cerebrospinal fluids), where the albumin concentration is too low to be detected by the dye-binding assays [12,13]. This includes the various formats of enzyme-linked immunosorbent assay (ELISA).

In this study, we report a fluorescence (FL) immunoassay system that can measure albumin concentration in whole blood without centrifugation or dilution prior to measurement. Basically, the assay system takes advantage of the inherent simplicity of an immunochromatography assay (ICA) [14], while providing quantification of albumin concentration with a one-step simple operation in <12 min. Unlike the traditional lateral-flow ICA, fluorophore dye is used as a tracer molecule for the study. The assay system consists of an ICA test strip in a disposable cartridge, a FL-labeled detector buffer and a laser-FL scanner [15,16]. We evaluated the technical performances of the fluorescence immunochromatography assay (FL-ICA) system including linearity, recovery and precision. We also examined the comparability between the developed assay system and an automated BCG dye-binding method.

2. Materials and methods

We obtained HSA (A8763), rabbit IgG (I5006), anti-rabbit IgG (anti-RIgG, R4880), polyethyleneglycol, sodium azide, ammonium sulfate and sodium bicarbonate from Sigma (St. Louis, MO), Sephadex G25 and protein G, and activated Alexa Flour 647 from Amersham Pharmacia Biotech (Piscataway, NJ) and Molecular Probes (Eugene, OR), respectively. We obtained a 96-well microtiter plate from Greiner (Frikenhausen, Germany), nitrocellulose membrane (NC) from Millipore (Watertown, MA) and Cytosep for a sample pad from Pall (New York, NY). We obtained absorption pad from Schleicher and Schuell (Dassel, Germany).

2.1. Production of anti-HSA-antibody

We used HSA as an immunogen to produce mouse monoclonal antibody (mAb). Immunizations, cell fusion and the screening of hybridoma cells that pro-

duced anti-HSA-mAb were conducted according to a standard method [15,17]. First, we froze the positive clones confirmed by ELISA, and then thawed them to prepare for further selection. Usually, One-third of the clones did not survive this process. We selected anti-HSA-mAbs through two successive limiting dilutions. To generate ascitic fluid for a large-scale production of mAb, we injected hybridoma cells into the peritoneal cavity of mice. We purified the mAb from ascitic fluid a series of purification steps: membrane ultrafiltration–ammonium sulfate precipitation–protein G column. Then, we eluted the mAb from the column with 100 mmol/l glycine–HCl (pH 2.5), neutralized with 0.1 vol. of 1 mol/l Tris (pH 8.0) and stored it at − 70 °C until it was ready to use.

2.2. ELISA

For ELISA, we incubated a 96-well microtiter plate with 50 μl of the antigen at the concentration of 2 μg/ml in a 50 mmol/l carbonate buffer (pH 9.6) overnight at 4 °C. The plate was blocked for 30 min at 37 °C in a blocking solution (3% BSA in PBS). After the plate was incubated with culture supernatants for 1 h at 37 °C, it was washed extensively three times in PBS-Tween 20 (PBST) for 10 min, and then incubated for approximately 1 h at room temperature with HRP-conjugated goat anti-mouse IgG. Following the final rinse with PBST, antibody was visualized with 50 μl of a TMB substrate solution and the reaction was stopped by adding 50 μl of 10 mmol/l H_2SO_4. The absorbance was measured at 450 nm in an automatic ELISA reader (Bio-Rad model 550).

2.3. Labeling of antibody/antigen with FL dye

To label the HSA with FL dye, we mixed 10 μl of a 1 mol/l sodium bicarbonate buffer (pH 8.3) with 100 μl of purified HSA (1 mg/l), followed by adding 1 μl of activated Alexa Fluor 647 (10 mg/ml). After incubating the mixture overnight at 4 °C, we applied the mixture onto a Sephadex G25 chromatography column to separate the free Alexa dye from the conjugates. We collected the FL-labeled HSA as elutes by centrifuging the column at 2500 rpm for 2 min. We labeled the anti-RIgG/anti-HSA-mAb with FL dye and purified the conjugates as noted above. To make a detector buffer, we mixed fluorescence-labeled human serum albumin (HSA-FL) and fluorescence-labeled anti-rabbit IgG (anti-RIgG-FL), and fluorescence-labeled anti-HSA monoclonal antibody (anti-HSA-FL) and anti-RIgG-FL at the proper concentration for the competition assay system and for the inhibition assay system, respectively (Table 1). We stored the mixed detector buffer at 4 °C for immediate use or lyophilized and stored it at − 70 °C for an extended storage.

2.4. FL-ICA strip, cartridge and instrument

We prepared the assay test strips in the laboratory to fit into a disposable cartridge. For both the sample and the absorption pad, we cut cellulose sheets, Cytosep and 470, into sizes of 4 × 20 mm. We assembled the pads with antibody/antigen-immobilized NC on a laminate polystyrene card. As an internal standard, we coated the control line with rabbit IgG (1 mg/ml). And the test line was coated with anti-HSA-mAb (capture Ab, 3 mg/ml) or HSA antigen (capture Ag, 5 mg/ml) using a BioJet dispenser (BioDot, Irvine, CA) in 1 mm wide line (Table 1). We kept the strip card in a dry vacuum chamber overnight prior to placing it into a plastic disposable cartridge (15 × 90 mm). Once we assembled the cartridge, we immediately sealed it in a foil pouch with a desiccant and then kept it at room temperature. To quantify the distribution of the FL signal on the window of cartridge, we developed a one-dimensional FL scanner. We describe details of the system components and the principle of the laser-FL scanner elsewhere [15,16].

Table 1
Comparison of two assay systems

	Antibody-immobilized system (competition type)		Antigen-immobilized system (inhibition type)	
Control line	RIgG	1	RIgG	1
Test line (mg/ml)	anti-HSA-mAb	3	HSA	5
Detector (μg/ml)	HSA-FL	36	anti-HSA-FL	43
	anti-RIgG-FL	0.23	anti-RIgG-FL	0.23

2.5. Assay procedure

For optimal conditions, we used pooled serum from five healthy individuals as standard material in FL-ICA. The albumin level of the pooled serum was 40 g/l. For a lower level of serum, we diluted the serum with PBS containing 3% gelatin; for a higher level of serum, we spiked the serum with HSA. The detector buffer was a mixture of Alexa 647-labeled HSA (36 μg/ml) and anti-RIgG (internal-control detector Ab, 0.23 μg/ml) or Alexa 647-labeled anti-HSA-mAb (43 μg/ml) and anti-RIgG in PBST. We added 5 μl of serum or 10 μl of whole blood to 70 μl of detector buffer and loaded the mixture onto the sample well of the cartridge. After a 10-min incubation, we inserted the cartridge and scanned it with the laser-FL scanner. Using Gaussian fitting, we converted the relative fluorescence units (RFU) at the test and the control line into the area value (test: A_T, control: A_C). We plotted the area ratio of A_T/A_C against the concentration of HSA. We obtained the equation for the calibration curve and the correlation of coefficient (r) using a Microsoft Excel program.

2.6. Method comparison

We collected the whole blood samples to compare the assay systems from 90 normal subjects and 30 patients with liver disease. They had visited Hallym University Medical Center in Chuncheon, Korea. After fully explaining the nature and possible consequences of the studies, we obtained informed consent from the study participants. We analyzed the separated serum samples in side-by-side assays using a BCG method (Hitachi 747) and the FL-ICA method to measure the concentration of HSA. We compared the results with the Bland–Altman difference plot analysis and Passing–Bablok method using the Medcalc version 6.12 software program (Medcalc, Mariaekerke, Belgium) [18].

3. Results

3.1. Production and characterization of mAbs

To select specific mAbs, we screened supernatants from hybridoma cells against HSA by ELISA

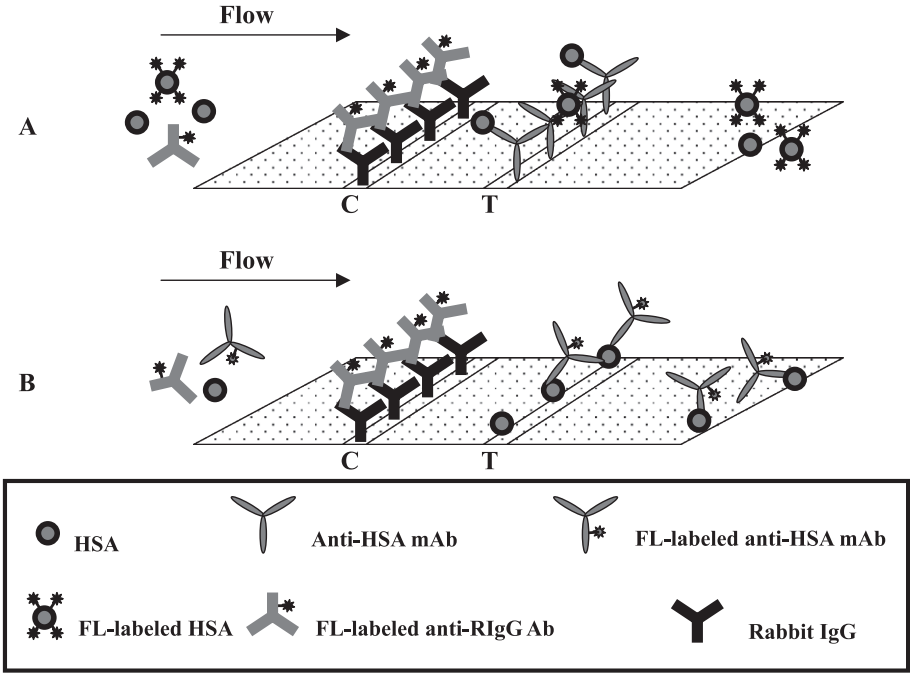

Fig. 1. The components and their interactions involved in the two FL-ICA systems. C and T in A, B are the control and test lines. In the competition assay system (A), the anti-HSA-mAb and the RIgG were immobilized on the test and the control lines, respectively. The HSA-FL competes with the HSA in sample for binding to immobilized antibody. In the inhibition assay system (B), the HSA and the RIgG were immobilized on the test and control lines, respectively. The HSA in the sample inhibits anti-HSA-FL in the detector buffer for binding to immobilized HSA.

and selected 12 positive clones. Serum proteins were separated by SDS-PAGE, electrotransferred to NC and immunoblotted with the mAbs to check the specificity [19]. All of the antibodies specifically recognized a single protein band of 68 kDa on the gel, which corresponded to the molecular size of HSA (data not shown). The clone 22C5 showed the highest signal by both ELISA and immunoblot, and it was used for a detector or a capture Ab throughout this study.

3.2. FL-ICA

To quantify albumin in this study, we designed two FL-ICA systems: one for a competition assay system (Fig. 1A) and the other for an inhibition assay system (Fig. 1B). Both systems adopted the principle of traditional lateral-flow ICA to analyze HSA. The test strip was composed of some basic components used in the conventional strips, which includes a sample pad to apply sample, a NC membrane to separate and detect analytes, and an absorption pad to generate capillary action. One major difference between the FL-ICA system and the conventional ICA is that fluorescence dye was used to detect analytes and integrated into the buffer. In the FL-ICA system, the higher the concentration of HSA in the blood, the less accumulation of the detector antibody/antigen on the test line. Thus, the FL intensity at the test line is inversely related to the amount of HSA in the blood. Another protein irrelevant to HSA, rabbit IgG was fixed at the control line to confirm that the sample goes through the lines and that the test works properly.

We tested the competition FL-ICA system over a range of normal concentrations of serum albumin. We quantified the intensity of FL on the test strip after the competitive immune reaction by scanning it with a laser-FL scanner. Fig. 2 shows a profile of RFU as a pattern of peaks graphed from the scanned raw data. The first peak (a) and the second peak (b) display RFU on the control line and on the test line, respectively, as plotted in the *y*-axis. We plotted arbitrary distance from the sample well of the cartridge in the

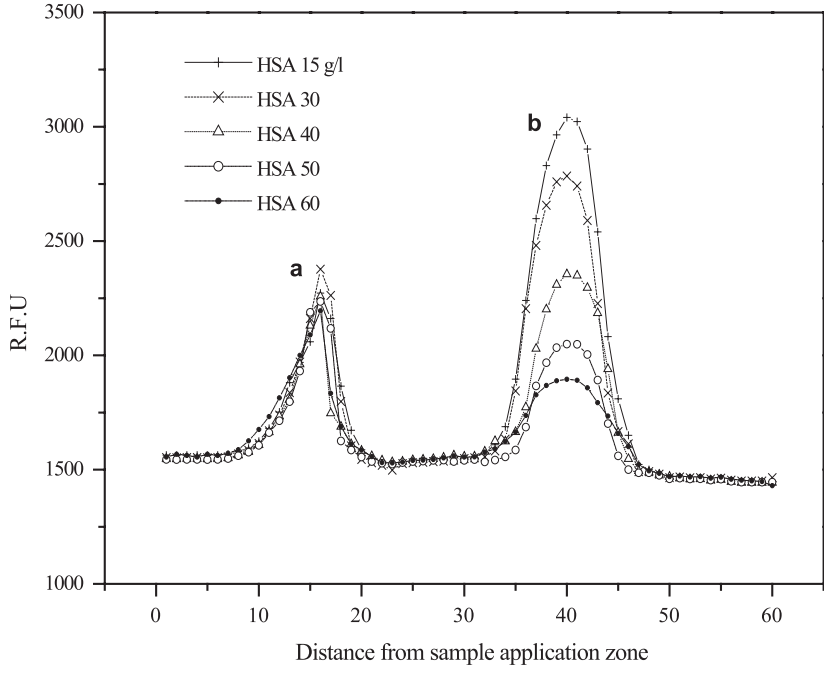

Fig. 2. The scanning profile of relative fluorescence units in a competition assay system. The cartridge loaded with sample containing a given concentration of albumin was inserted and scanned in the laser-FL scanner. a and b show the relative fluorescence units on the control and test lines depending on the albumin concentrations in the samples, respectively.

x-axis. The RFU of the first peaks (a) on the control lines was almost unchanged even with a big difference in the albumin concentration of the samples. This finding indicates that the interaction between rabbit IgG and anti-rabbit IgG is independent of albumin concentrations. Thus, it shows that anti-RIgG-FL in the detector buffer is functioning as a good internal standard. In contrast, the RFU of the second peaks (b) on the test lines changed according to the albumin concentration in the samples. As the albumin concentration in the samples increased, the RFU decreased considerably. We observed an identical pattern of RFU profiles with the inhibition FL-ICA system. We observed this pattern even though the difference in RFU was not very noticeable at the high albumin concentration in the samples (>35 g/l) as compared to that of the antibody-immobilized system (data not known). These results demonstrated that the two ways formatted the FL-ICA systems work accordingly and can be used as a tool to quantify albumin in serum.

3.3. Calibration curves of FL-ICA

We first tested the competition assay system for HSA. We obtained the calibration curve by comparing the area ratio (A_T/A_C) and the albumin concentration in the samples. The profile of RFU at each line was converted to A_T/A_C and plotted at the *y*-axis against the albumin concentration at the *x*-axis. We observed a reliable correlation of coefficient (r) in the antibody-immobilized FL-ICA system between the two parameters ($r = 0.998$), and an excellent linearity was displayed throughout the entire measuring range (Fig. 3A). The coefficients of variation (CVs) from the area ratio (A_T/A_C) of five independent experiments at each concentration was $< 10\%$.

We set up another FL-ICA system by changing an experimental format from the competition type to the inhibition type. HSA was immobilized on the test line instead of the antibody. HSA-immobilized inhibition FL-ICA proved to be less sensitive to a higher albumin concentration as compared to that of the antibody-immobilized competition assay (Fig. 3B). However, the inhibition FL-ICA still showed a good correlation between the A_T/A_C and the albumin concentration ($r = 0.998$) and the CV was $< 10\%$ at each albumin concentration tested.

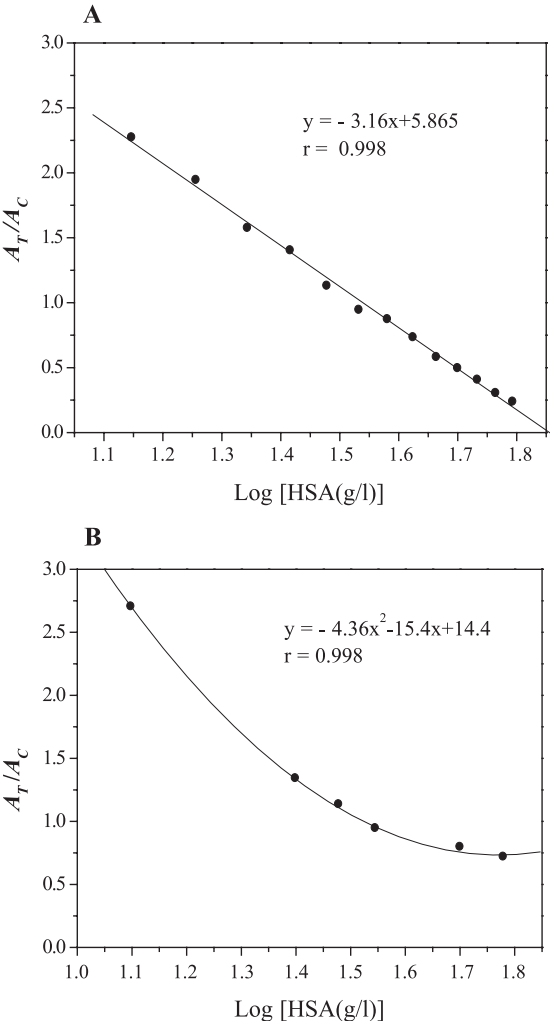

Fig. 3. Calibration curves for the competition assay (A) and the inhibition assay system (B). The area ratio (A_T/A_C) converted from the relative fluorescence units is plotted against the concentration of HSA in the sample. The correlation coefficient (r) between the area ratio and the concentration of HSA are shown in the antibody-immobilized (A) and in the antigen-immobilized FL-ICA system (B). The spiked points for calibration curves in A and B were obtained as the mean of 5 independent experiments at each albumin concentration.

3.4. Performance test for FL-ICA

We conducted imprecision and recovery tests to evaluate the analytical performance of the competition FL-ICA system with serum reference material. We prepared 4 control samples covering the con-

centration range of HSA that is usually encountered in clinical practice (Table 2). For the within-run imprecision, we performed 20 replicate tests of each control sample. We conducted the between-run assay on 10 sequential days, with 10 runs/day and with 10 replicate cartridges at each albumin concentration. The CVs of within- and between-run assay in the new immunoassay system were, respectively, 7.3% and 3.6% at 12.2 g/l, 5.0% and 6.9% at 24.5 g/l, 4.7% and 3.2% at 37.9 g/l, and 4.9% and 4.8% at 60 g/l. The analytical recovery was calculated from the same four controls. After testing the samples in 10 replicates in one analytical run, we found that the measured values were within 5% of the expected values in each control sample. These results demonstrate that the new FL assay provides a confident performance compared to other well-known assay systems.

To confirm the reliability of the immunoassay, we also analyzed the linearity in a series of parallel dilutions using the known albumin concentration of the Lyphocheck (Bio-Rad, #14070). The results from the FL-ICA system corresponded well to the expected values from the Lyphocheck albumin as shown in Fig. 4. 0.986 was the correlation of determination (r) of the albumin concentration between the new assay and the Lyphocheck.

3.5. Comparison of the FL-ICA and the BCG method

We compared the FL-ICA with the BCG dye-binding method of a Hitachi 747 biochemical analyzer. We observed an acceptable agreement between the two methods over the clinical relevant range of HSA concentrations. We made these observations using the

Table 2
Imprecision and recovery of the FL-ICA system

HSA (g/l)	Within-run ($n=20$)			Between-run ($n=10$)[a]			Recovery ($n=10$)
	Mean	S.D.	CV (%)	Mean	S.D.	CV (%)	
12.2	11.8	0.9	7.3	12.1	0.4	3.6	97.8%
24.5	24.3	1.2	5.0	24.0	1.6	6.9	101.3%
37.9	39.5	1.9	4.7	38.5	1.2	3.2	104.5%
60	57.3	2.8	4.9	55.9	2.7	4.8	96.3%

[a] Between-run assay was conducted on 10 sequential days, with 10 runs/day and with 10 replicate cartridges at each albumin concentration.

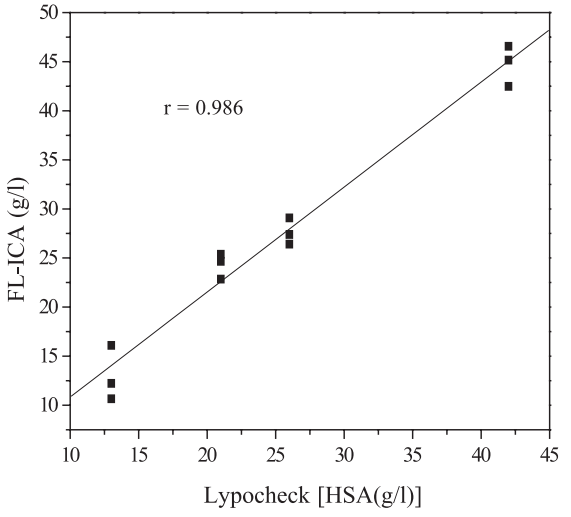

Fig. 4. Linearity of the antibody-immobilized competition FL-ICA system to the HSA concentration. We measured known albumin concentrations of Lyphocheck Levels 1 and 2 from the FL-ICA by 2 diluting the serial two-fold. The linear curve and the correlation of determination (r) are shown.

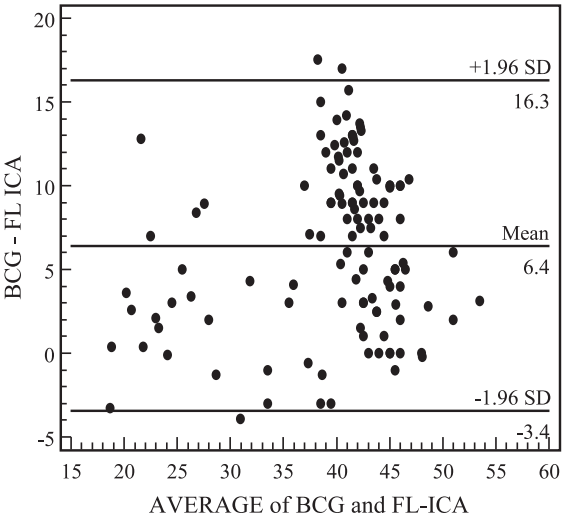

Fig. 5. Comparison of the BCG dye-binding method (Hitachi 747) and the FL-ICA by Bland–Altman difference plot analysis. Clinical samples ($n=120$) were analyzed using side-by-side assays and the difference between the two values (BCG-FL) in the y-axis is plotted against the mean value in the x-axis. The mean difference ± 1.96 S.D. is shown. The regression analysis by Passing–Bablok yielded a slope of 0.98 (95% confidence interval, 0.79–1.12) and a y-intercept of 7.55 g/l (95% confidence interval, 2.4–14.2 g/l).

Bland–Altman difference plot analysis as shown in Fig. 5. Horizontal lines were drawn at the mean difference and, at the mean difference ± 1.96 times, the standard deviation of the differences. Among 120 samples, only 3 samples were out of interchangeable range between 2 methods. The maximum difference between the results of the BCG-binding and the immunoassay varied from −3.4 to 16.3 g/l, with a mean difference (S.D.) of 6.4 g/l. According to Passing–Bablok method, the regression analysis yielded a slope of 0.98 (95% confidence interval, 0.79–1.12) and a *y*-intercept of 7.55 g/l (95% confidence interval, 2.4–14.2 g/l). This finding indicates that there is a statistically good correlation between two methods ($p < 0.001$).

3.6. Measurement of HSA in whole blood

We determined the correlation of HSA concentration between serum and whole blood from the same individual. We drew 5 ml of whole blood from the patients who had various gastrointestinal diseases. Each sample was divided into two tubes, and one was used for measuring the HSA concentration in whole blood. The other was incubated for clotting, centrifuged and then used for measuring the serum HSA concentration. We compared the concentration of 5 μl of serum with those of 5, 10, 15 and 20 μl of whole blood in the laser-FL scanner (data not shown). The HSA concentration in 10 μl of whole blood corresponded well to that in 5 μl of serum, as shown in Fig. 6. The correlation of coefficient (*r*) obtained was 0.945, with the slope of 1.005 from the calculated equation.

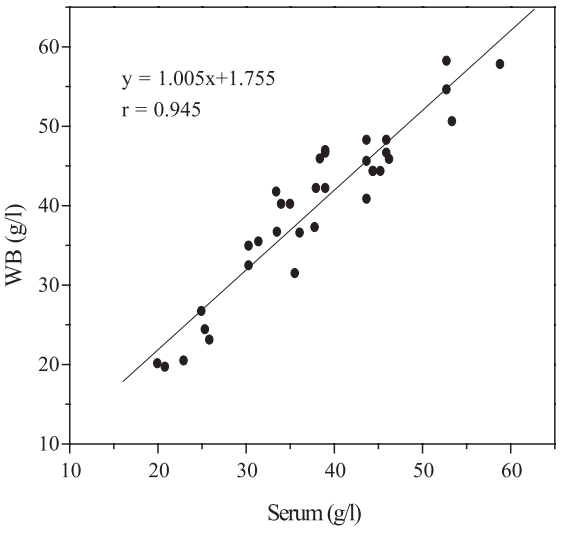

Fig. 6. Comparison of albumin concentrations in serum and in whole blood with the FL-ICA. Concentration of HSA in 5 μl of serum is compared and plotted against 10 μl of whole blood from same sample ($r = 0.945$).

4. Discussion

In the present study, we developed two FL-ICA systems, a competition- and an inhibition assay type, to quantify human albumin in serum and in whole blood. We modified the FL-ICA systems from a traditional lateral-flow immunochromatography using FL dye as a tagging molecule to measure an analyte in the sample. Both of the assay systems showed a good analytical performance with a wide range of albumin concentration in the sample.

A current trend in quantifying albumin is to use dye-binding methods for serum and turbidimetry/nephelometry for urine with automated analyzers [20]. Kessler et al. [21] introduced a laser-induced FL system as the non-immunological assay of albumin in urine. However, their system was based on the probe Albumin Blue 670, which becomes highly fluorescent on binding to albumin, and was coupled to a complicated flow-injection analysis system. Recently, researchers have developed competitive immunoassays based on capillary electrophoresis with laser-induced FL. These immunoassays determine the varieties of analytes in biological mixtures, and they include immunoassays of human serum albumin [22,23]. The assays possess the following four characteristics: (1) a short analysis time, (2) a small amount of sample, (3) high detection sensitivity and (4) the capability for simultaneous analysis of multi-components. These assays have considerable potential for use in areas of clinical chemistry since these methods can be remarkably miniaturized in the future. However, improvements still need to be made in the point of sample preparation, trained personnel to handle them, expenses, and in other areas. In contrast, our HSA immunoassay system is easy to use, inexpensive, requires no expert operation and especially

works with whole blood. More importantly, the FL-ICA system corresponds well to the popular dye-binding method as shown in Fig. 5.

Despite their specificity, immunochemical assays are not routine methods for measuring serum albumin. Compared to the dye-binding assays, the immunological methods are relatively expensive to administer. Thus, instrument manufacturers are reluctant to incorporate the immunoassays in clinical analyzers. They regard it as more appropriate for tests beyond the detection limit of the dye-binding assays, such as with urine.

When the FL-ICA method in the competition assay format was compared with the BCG dye-binding method from 120 clinical serum samples, the difference between the methods was within the mean $\pm$ 1.96 S.D. This finding indicated that the two methods could be used interchangeably. The regression analysis according to Passing–Bablok also showed a statistically good correlation between the two methods. However, there has been a report that the BCG dye-binding method correlated poorly with an immunological method [24]. When we tested the reliability of the FL-ICA system by comparing the standard material of Lyphocheck, we observed a good correlation. The reproducibility of the FL-ICA system displays no differences from well-known assay systems and is practically applicable. Taken together, these results indicate that the competition FL-ICA system provides confident performance characteristics for quantifying albumin in serum. The inhibition FL-ICA system showed less sensitivity to a normal range of albumin level, but the slope of the calibration curve in a low-level range was much steeper than that of the competition FL-ICA system. In general, the albumin level of serum falls at an acute-phase reaction in response to any of a number of stressful situations, such as infection, inflammation, surgery, a burn or even injection of phenobarbital [25]. Thus, the inhibition FL-ICA appears to be a useful system to confirm an acute-phase reaction.

While the dye-binding assay systems have their own advantages, they are still inconvenient since they require time-consuming procedures, relatively expensive equipment, and a qualified expert to administer the method. One major advantage of the FL-ICA over the ELISA is that the method does not require washing steps [26]. In ELISA, several washing steps are required to remove an unbound analyte and a detector antibody. The FL-ICA system can quantify the albumin level in whole blood without centrifugation and in serum without dilution of the sample. Since the FL-ICA system needs only a small volume of sample, uses a portable laser FL scanner and processes the test in 12 min, it is very suitable for point-of-care testing of human albumin. Presently, we are devising a FL-ICA to quantify urinary albumin, which is an essential screening for hypertension and diabetes patients [27].

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