

Establishment of a Novel Quantum Dots-Encoded Microbead-Based Flow Cytometric Method for Quantification of Soluble FcεRIα in Serum

Kun Lin,¹ Wen Xu,¹ Wanwan Li,² Yuankui Leng,² Weijie Wu,² Xia Peng,¹ Yuting Liang,¹ Li Li^{1*}

¹Department of Laboratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200080, People's Republic of China

²State Key Lab of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, People's Republic of China

Received 10 January 2017; Revised 16 March 2017; Accepted 13 April 2017

Grant sponsor: National Natural Science Foundation of China, Grant numbers: 81401335, 81471593, 81273276

Grant sponsor: Clinical Auxiliary Departments Construction Project of Shanghai Shen-Kang Hospital Development Center, Grant number: SHDC22014005

Grant sponsor: Science and Technology Committee of Shanghai, Grant numbers: 14411963200, 15431901900

Grant sponsor: Shanghai Jiao Tong University Medical-Engineering Cross Youth Project, Grant number: YG2015QN15

Additional supporting information may be found in the online version of this article.

*Correspondence to: Li Li, Department of Laboratory Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, People's Republic of China.
Email: annylish@126.com

Abstract

The soluble form of the transmembrane glycoprotein, FcεRIα which corresponds to the high-affinity receptor for IgE, is found in serum. Growing evidence suggests the pathogenic role of IgE and FcεRI in systemic lupus erythematosus (SLE). The goal of this study is to develop a sensitive and standardized cytometric assay for quantification of sFcεRIα. A membrane emulsification technique was utilized to incorporate CuInS₂/ZnS quantum dots and Fe₃O₄ nanoparticles into poly (styrene-co-maleic anhydride) microbeads. The beads were then carboxylated and coated with capture antibody monoclonal anti-human FcεRIα. This antibody binds to FcεRIα but does not block the binding of FcεRIα to IgE. After incubation with standards or serum samples, the microbeads were incubated with excessive native human IgE, followed by incubation with Phycoerythrin (PE) conjugated anti-human IgE. The resulting quantum dot microbeads were gated, and sFcεRIα quantification was analyzed based on PE fluorescence intensity. The method exhibited good linearity ($R^2 > 0.99$), and the limit of detection was established at 0.29 ng/mL with the dynamic range of up to 200 ng/mL. The precision of the assay validated by intra- and inter-assay variability met the acceptance criteria with the mean recovery falling within 80–110% of the theoretical concentration and a corresponding CV < 20%. We tested 149 serum samples which 89 were from SLE patients and 60 were from healthy volunteers. For the first time, we detected an increased sFcεRIα level in the serum of SLE patients, which was confirmed by a commercial ELISA kit. Compared to ELISA, this novel method is more sensitive and efficient. It allows for the simple comparative analysis of sFcεRIα levels in health and disease. © 2017 International Society for Advancement of Cytometry

Key terms

quantum dots-encoded microbeads; flow cytometry; sFcεRIα; cytometric bead assay; SLE

INTRODUCTION

FcεRI, the high affinity receptor for immunoglobulin E (IgE), is a tetrameric receptor constitutively expressed on mast cells and basophils. It consists of an alpha-chain, a beta-chain and a gamma-chain dimer. The alpha-chain is an approximately 60 kDa transmembrane glycoprotein, and its extracellular domain binds to the Fc region of IgE. The beta-chain amplifies the signal transduction of the gamma-chain (1). There is another trimeric isoform of FcεRI which lacks the beta-chain. This isoform can be found on the surface of monocytes, eosinophils and dendritic cells (2,3). The concentration of serum IgE is the lowest of all immunoglobulins. However, FcεRI has the highest affinity for its ligand IgE of all the immunoglobulin receptors (4). FcεRI plays an important role in the capture, clearance/endocytosis and regulation of serum IgE

level in vivo (5). Crosslinking of FcεRI by allergen binding to FcεRI-bound IgE leads to mast cell degranulation and the release of inflammatory mediators (i.e., histamine), which is responsible for initiating allergic response.

The soluble low-affinity IgE receptor CD23 (also known as FcεRII) has made its way into clinical practice as a biomarker in chronic lymphocytic leukemia. And galectin-3, a secretory low-affinity IgE-binding protein, is also found to be upregulated in various types of autoimmune diseases and cancers (6). Over recent years, studies have proved that the soluble form of FcεRI, namely sFcεRI, circulates in serum (7). sFcεRI is a truncated alpha-chain containing one intact IgE-binding site while lacking the transmembrane region and the cytoplasmic domain. Thus, its molecular weight is about 40 kDa comparing to the full length. It has not yet been shown how sFcεRI is produced and what cell types can release this protein in vivo. sFcεRIα can form a IgE-sFcεRIα complex in serum. It has been shown that sFcεRIα can block IgE binding to the cell surface of FcεRI, exert the potential to reduce free IgE level, and alleviate allergic response (8,9). The studies of sFcεRI first emerged in the 1990s (10). However, later studies focused on the therapeutic value of sFcεRIα but neglected its importance as a biomarker (11). It was proven that cell surface FcεRIα expression correlated with serum IgE in various diseases (12). In addition, in vitro studies showed that sFcεRIα can be generated after FcεRI crosslinking-induced receptor activation through a post-translational modification mechanism (7,13). These findings indicate that sFcεRIα may be a potential biomarker for IgE-mediated immune responses.

IgE antibodies not only provide protective immunity against parasitic infections but also are the center of allergic reactions. Recent studies further expand the known pathogenic mechanisms of IgE-mediated inflammation and elucidate a role for IgE in autoimmunity. IgE antibodies specific for the bullous pemphigoid (BP) autoantigen, BP180, are detected in sera and biopsy samples from the majority of BP patients (14). In systemic lupus erythematosus (SLE), the concentration of dsDNA-specific IgE autoantibodies found in patient serum correlated with the disease severity. They could activate plasmacytoid dendritic cells by triggering phagocytosis via FcεRI receptor (15). In addition, autoantibodies against FcεRIα are generated in autoimmune diseases, some of which can initiate degranulation (16,17). Since FcεRI is so widely recognized as critical receptor participating in IgE-associated immune responses, more detailed studies investigating the possible link between serum sFcεRIα to clinical symptoms of autoimmunity are necessary. Therefore, it is critical to develop a sensitive and high-throughput diagnostic platform for sFcεRIα quantification.

Herein, we described for the first time a novel magnetic quantum dots (QDs)-encoded microbead-based flow cytometric method for detection of sFcεRIα. Highly fluorescent CuInS₂/ZnS QDs and superparamagnetic Fe₃O₄ nanoparticles were co-incorporated into polystyrene-co-maleic anhydride (PSMA) to create microbeads. The immunomagnetic cytometric QDs-encoded microbead assay was significantly more sensitive and stable than the traditional ELISA method (18). Moreover, our cytometric method possessed a wider linear range. Based on this, we explored the sFcεRIα concentration and its correlation with serum IgE in SLE patients. Technical validations of the assay showed good performance when dealing with serum samples. This method can be implemented routinely on flow cytometry and would be a very convenient technology for sFcεRIα quantification.

MATERIALS AND METHODS

Reagents

Copper (I) iodide, zinc stearate (chemical grade), trichloromethane (analytical grade), alcohol (analytical grade), toluene (analytical grade), sodium dodecyl sulfate (SDS, analytical grade), hydrochloric acid (HCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. Indium (II) acetate, 1-octadecene (ODE, technical grade, 90%), 1-dodecanethiol (DDT, >98%), EDC, S-NHS, poly (styrene-co-maleic anhydride) (PSMA, MW = 224,000, 7 wt% anhydride) were purchased from Sigma Aldrich. SPG glass membrane (tubular porous, 4.9 pore size) was purchased from SPG Technology, Japan. Fe₃O₄ nanoparticles were provided by GoldMag Bio Technology Co., Ltd., China. Anti-human Fc-epsilon Receptor I alpha monoclonal antibody (clone AER-37/CRA1, Cat#14-5899) and Phycoerythrin (PE) conjugated anti-Human IgE (Cat#12-6986) were purchased from eBioscience. Native human IgE from myeloma plasma was purchased from Merck Millipore (Cat#401152). Recombinant human Fc epsilon RI alpha protein was purchased from R&D systems (Cat#6678-FC-050). Human Fc epsilon RI Platinum ELISA kit was purchased from affymetrix (Cat#BMS2101).

Patients and Controls

This method was validated on 149 serum samples consisting of 89 samples from SLE patients and 60 healthy volunteers. Consecutive hospitalized SLE patients were recruited from Shanghai General Hospital between October 2015 and July 2016. SLE was diagnosed according to the 1982 revised criteria for the classification of SLE (19). Healthy volunteers were randomly selected from physical examination center during the same period. The controls had normal liver and kidney function, and their fasting blood glucose and lipid levels were within the reference range. The general characteristics of the SLE patients and the controls are summarized in Table 1. We

Table 1. Characteristics of SLE patients and controls

	N (PATIENTS)	SEX (MALE/FEMALE)	AGE
SLE	89	8/81	35.0 (25.0, 52.0)
Controls	60	11/49	35.5 (22.0, 55.8)

excluded participants with additional complications including other autoimmune diseases, malignancies, viral hepatitis, and patients who received heparin treatment. All participants signed a written consent and the study was approved by the medical ethics council of Shanghai General Hospital. Blood samples were collected in coagulation-promoting tubes, and serum samples were separated and stored at -80°C . The clinicopathological details of patients were collected including age, gender, and test results of circulating autoantibodies, such as ANA, anti-dsDNA, and anti-Sm.

Synthesis of Quantum Dots-Encoded Microbeads

NIR-emitting $\text{CuInS}_2/\text{ZnS}$ core/shell nanocrystals were synthesized from Copper (I) iodide, Indium (II) acetate and zinc stearate as starting compounds according to the Reiss lab protocol with some modifications (20,21). Then the prepared $\text{CuInS}_2/\text{ZnS}$ core/shell nanocrystals were incorporated together into microbeads with magnetic nanoparticles via the membrane emulsification technique. The oil phase was prepared by dissolving a certain amount of $\text{CuInS}_2/\text{ZnS}$ QDs, magnetic nanoparticles and PSMA in 8 mL toluene. The aqueous, continuous phase was 200 mL water consisting with 0.5% SDS as stabilizer. A SPG glass membrane was installed into a SPG emulsification device (Japan's SPG technology Co., Ltd.). The dispersed oil phase was stored in a tank connected to a gas inlet, and then pressed into the continuous phase to form droplets by pushing through the pores of the SPG membrane wall with nitrogen gas. Uniform droplets were produced under an adequate gas pressure and stabilized with emulsifiers dissolved in the continuous phase. During droplet formation, the continuous phase was gently stirred to prevent creaming of the droplets. After that, the emulsion was stirred for 24 h to evaporate the toluene. During the evaporation process, the droplets gradually solidified into PSMA microbeads containing hydrophobic QDs and magnetic nanoparticles. Finally, the microbeads were centrifuged, washed with pure water and ethanol (separately) three times, and then collected by lyophilization. The carboxylation of the PSMA microbeads was performed by hydrolyzing the anhydride groups on the microbead surface. Approximately 0.1 g of the QDs-encoded microbeads were suspended in 15 mL HCl aqueous solution ($\text{pH} = 1$) and stirred for 24 h. The resulting carboxylated microbeads were washed with a water/ethanol mixture (1:1 volume ratio) to remove excess HCl. They were collected and stored at 4°C (22).

Cytometric Bead Immunoassay of sFcεRIα

To develop a cytometric bead assay for quantification of sFcεRIα, we selected a monoclonal antibody CRA1 that would recognize the extracellular domain of FcεRIα without blocking

the binding of sFcεRIα to IgE. The coating of the microbeads with CRA1 was based on the previously published method (23). Human recombinant FcεRIα was used for constructing the standard curve. Several experimental conditions were evaluated for achieving optimal stability of the antigen-antibody and receptor-ligand complex. We carefully investigated the effects of the amount of probe antibody coating, temperature (4, 25, or 37°C), incubation time, composition of the assay buffer, and blocking steps. The experimental procedures described below were considered optimal conditions for the assay. These conditions resulted in the highest sensitivity and stability.

The coating of the QDs-encoded microbeads with capture antibody CRA1 was achieved by the amide bond between the carboxyl groups on the modified microbead surface and the primary amine groups of the protein. In brief, 3×10^5 microbeads were washed and suspended in 100 μL activation buffer followed by 10 μL fresh solution of EDC (50 mg/mL in activation buffer) and S-NHS (50 mg/mL in activation buffer) added, respectively. After incubation in the dark on a shaker for 20 min, excessive EDC and S-NHS were washed off with PBS. Next, activated microbeads were resuspended and adequate amounts of CRA1 were added. The reaction mixture was incubated at 4°C overnight on a shaker. Then, excessive antibody was removed with PBS. Microbeads coated with CRA1 were resuspended in blocking buffer (1% BSA/PBS) for 30 min at 25°C on a shaker. Thereafter, the coated microbeads were ready for further immunoassay.

As the first step of the immunoassay, 2×10^4 CRA1-coated QDs-encoded microbeads were added into each micro-well and incubated with standards or serum samples (100 μL /well) at 25°C for 1 h in a 96-well plate. After the incubation, beads were washed three times with wash buffer on a magnetic plate separator. In the second step, excessive IgE (200 ng/mL in assay buffer, 100 μL /well) was added. After 1 h incubation at 25°C , beads were washed again and incubated with detection antibody (PE conjugated goat anti-human IgE in assay buffer, 100 μL /well) for 1 h at room temperature. After washing steps, samples were suspended and analyzed by flow cytometry using a Beckman Coulter FC 500, equipped with 488 and 633 nm lasers. The beads were gated by size on FSC/SSC plot as well as by the fluorescence on two channels (BP 675/30 nm and BP 755/60 nm) on 488 nm laser excitation. For each assay, about 2,000 gated microbeads were acquired. The PE (BP 575/16 nm) signal was measured and the mean fluorescence intensity of PE was used for sFcεRIα quantification. Flow Cytometry data were analyzed by the CXP software (version 2.2, Beckman Coulter). All assays were repeated three times.

Technical Validation of the Assay

Linearity of calibration standards. Lyophilized human recombinant FcεRIα was reconstituted to prepare standard dilutions. For the standard curve, 12 gradient concentrations of FcεRIα ranging from 0.01 to 200 ng/mL were prepared in triplicates in the assay buffer. The R^2 of the standard curve

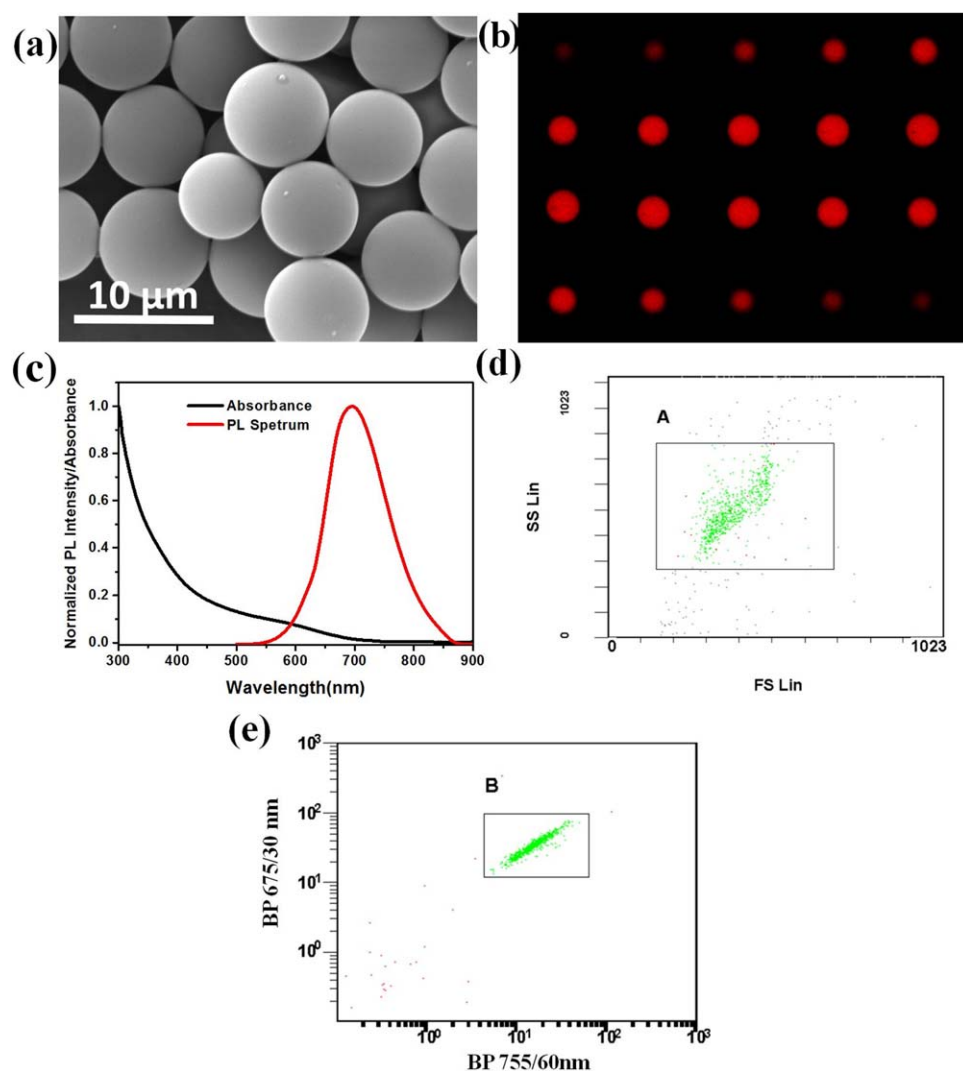


Figure 1. (a) SEM image of QDs-encoded microbeads; (b) confocal laser scanning microscopy luminescence images of sections from top to bottom through QDs-encoded microbeads; (c) absorbance and emission spectrum of CuInS₂/ZnS QDs; (d, e) performance of CuInS₂/ZnS QDs microbeads on FC 500. [Color figure can be viewed at wileyonlinelibrary.com]

was fitted using logistic regression algorithm calculated with OriginPro 8.5.

Sensitivity and specificity. The limit of blank (LoB) and the limit of detection (LoD) were determined according to the guideline EP17 published by Clinical and Laboratory Standards Institute (CLSI) (24). LoB was estimated daily by measuring six samples (B1–B6) with 10 duplicates for each sample over 10 days: B1, B2, and B3 were three serum samples with undetectable FcεRIα; B4 was the assay buffer; B5 was PBS and B6 was 1% BSA in PBS. $LoB = Mean_{blank} + 1.645 (SD_{blank})$. LoD was defined by measuring 40 replicates containing 0.4 ng/mL recombinant FcεRIα in five independent experiments. $LoD = LoB + 1.645 (SD_{low\ concentration})$. The specificity of the assay was assessed by omitting the coating antibody or replacing human FcεRIα with BSA.

Performance of reproducibility. Ten serum samples with nondetectable sFcεRIα were randomly selected, mixed, and diluted. Standard FcεRIα samples with four concentrations (5, 10, 50, and 100 ng/mL) were prepared in the diluted serum. Reproducibility was determined by performing intra- and inter-assay variability tests. For intra-assay tests, the four samples were analyzed eight times on the same plate. The inter-assay reproducibility was assessed by running the four identical samples on eight different plates by two independent operators. The precision of the method is qualified when the corresponding CV falls within 20%. The matrix interference was evaluated by recovery rate (%) which was calculated by dividing the sFcεRI concentration measured using the flow cytometer by the known concentration of standard FcεRIα in the sample. The acceptance criteria are met when the mean recovery falls within 70 and 110%.

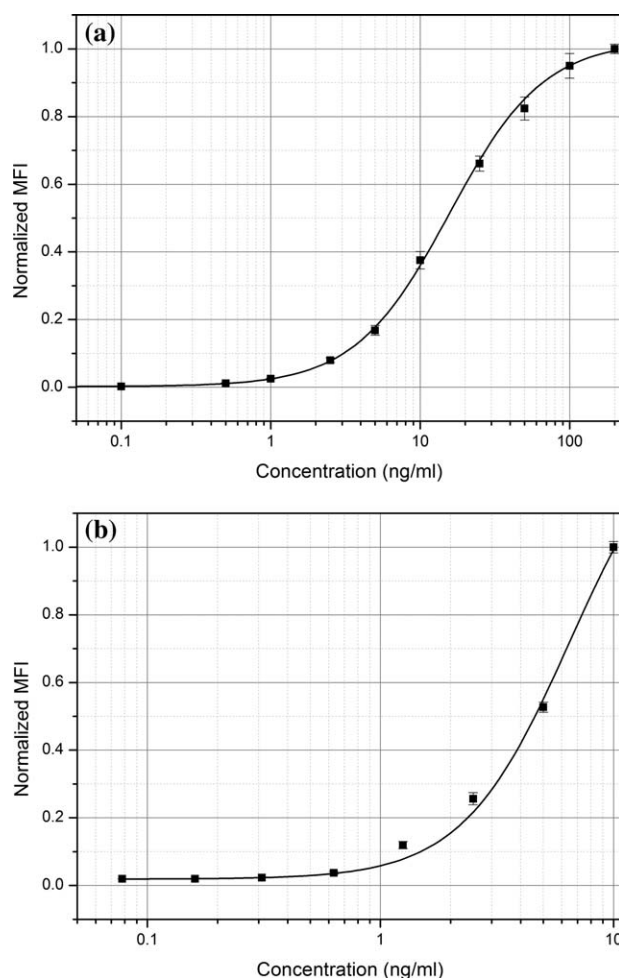


Figure 2. (a) A 12-point standard curve; (b) a 9-point standard curve of low concentration range.

Sample stability. The effect of different storage conditions on the sFcεRIα measurement was assessed according to the recovery of the standard recombinant FcεRIα initially added in the negative serum samples. Recombinant FcεRIα was added into negative serum samples at six different concentrations (5, 10, 25, 50, 100, and 200 ng/mL). The concentration of sFcεRIα in the samples was measured before and after three or more freeze-thaw cycles or stored overnight at 4°C. The recovery was calculated as the percentage of initial concentration.

Statistical Analysis

All statistical analyses were performed using PASW Statistics, version 18.0 and OriginPro.8.5. Normally distributed data were described by mean ± SD. Skewed data were described using median and interquartile range. When a normal distribution and equal variance were met between the two groups, a *t* test was used. Skewed data were log transformed before analysis to achieve a normal distribution. Non-parametric analysis or one-way ANOVA analysis was used to compare the sFcεRIα level among different groups. *P* values <0.05 were considered statistically significant.

Table 2. Determination of limits

	MEAN (NG/ML)	SD	CV (%)
Blank samples	0.1993	0.0041	2.06
low concentrations	0.3397	0.0529	15.58

RESULTS

Characterization of Quantum Dots-Encoded Microbeads

Uniform CuInS₂/ZnS QDs-encoded microbeads with a smooth surface were synthesized using SPG membrane technique. The diameter of the microbeads was about 7 μm. The CuInS₂/ZnS quantum dots were evenly distributed within the microbeads. The CuInS₂/ZnS QDs-encoded microbeads possessed excellent fluorescence stability even if there were changes in the external environment. Our NIR-emitting QDs-encoded microbeads were quite stable against temperatures ranging from 4 to 60°C and pH values ranging from 1 to 10. Moreover, the fluorescence intensity of the microbeads was stable over a 60-days storage at room temperature without extra protection, further guaranteeing their application in clinical detection (22). CuInS₂/ZnS quantum dots had a broad emission spectrum and can be detected in two fluorescence channels of flow cytometry. The characterization of the microbeads was summarized in Figure 1.

Performance Verification

Linearity of the assay. The 12 serial dilutions of reconstituted human FcεRIα (0, 0.01, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, and 200 ng/mL) were prepared in triplicates. The standard curve was shown in Figure 2a. The *R*² of the assay was 0.998. The assay exhibited good linearity even in low concentration range (0, 0.078, 0.16, 0.31, 0.63, 1.25, 2.5, 5, and 10 ng/mL), as depicted in Figure 2b. The *R*² of the assay was 0.991.

Determination of limits and specificity. LoB was determined at 0.21 ng/mL and LoD was determined at 0.29 ng/mL (see Table 2). The dynamic range for the assay was 0.29–200 ng/mL. Omitting the coating antibody CRA1 or replacing human FcεRIα with the same concentration of BSA resulted in a loss of the fluorescence signal over the whole range of concentrations.

Precision of the assay. First, the matrix effects of serum were evaluated by conducting parallel, serially diluted assays.

Table 3. Recovery of FcεRIα in diluted serum with different dilution factors

Serum dilution factors	1	2	5	10
FcεRIα amounts	5, 10, 50	5, 10, 50	5, 10, 50	5, 10, 50
Recovery	11.5%, 17.3%, 29.4%	20%, 46.1%, 55.9%	92.3%, 82.8%, 85.4%	80.7%, 88.2%, 110.8%

Table 4. Intra-assay variability

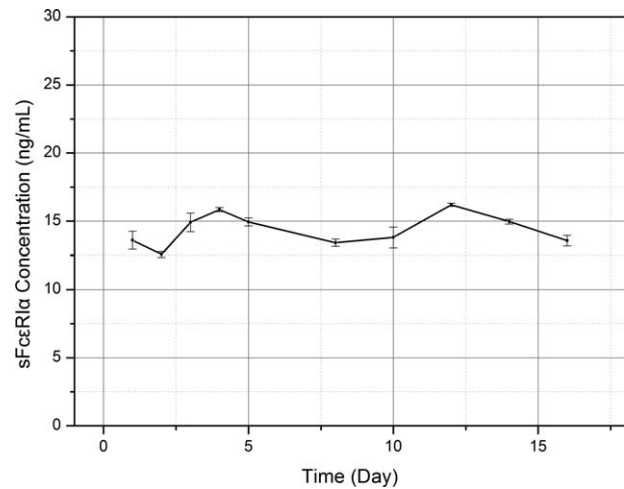
RSFcεRI (NG/ML)	MEAN	SD	CV (%)	RECOVERY (%)
5	4.8492	0.7033	14.5%	97.0%
10	10.3920	0.9331	8.98%	103.92%
50	51.9603	2.1809	4.20%	103.92%
100	83.6293	2.3499	2.8%	83.63%

Table 5. inter-assay variability

RSFcεRI (NG/ML)	MEAN	SD	CV	RECOVERY
5	4.9896	0.6352	12.73%	99.8%
10	9.9728	0.9302	9.33%	99.73%
50	49.4006	5.4768	11.09%	98.8%
100	81.3393	10.9464	13.46%	81.34%

Certain amounts of FcεRIα were added into assay buffer, a diluted mixture of serum samples containing nondetectable sFcεRIα by different dilution factors, and then detected by our method. The results showed that the recovery was in the range of 80–110% (see Table 3) when the serum dilution factor was ≥ 5 . Thus, all clinical serum samples were diluted five times prior to the assay. The reproducibility performance was shown in Tables 4 and 5 (from eight repeated experiments mentioned in “Materials and Methods”). Mean recovery was within 20% of the absolute concentration with a corresponding CV $< 20\%$ for all tested concentrations. These results met the acceptance criteria for immunoassay validation.

Sample and method stability. To assess the stability of sFcεRI in serum, six concentrations of FcεRI were analyzed before and after three and six freeze/thaw cycles. An additional aliquot was stored at 4°C overnight. Table 6 summarized the effects of different storage conditions on the stability of sFcεRI in serum. Storage at -20°C and freeze/thaw within three cycles prior to measurement did not significantly influence the outcome of sFcεRI concentrations. However, six freeze/thaw cycles or 4°C overnight had effects on the outcome. Furthermore, we evaluated the stability of antibody immobilized microbeads stored at 4°C by measuring the same aliquots of 15 ng/mL reconstituted FcεRIα over 16 days. The results were stable with the concentration at 14.38 ± 1.16 ng/mL and CV at 8.06% (Figure 3, $n = 10$).

**Figure 3.** sFcεRIα concentration measured by anti-human FcεRIα immobilized microbeads stored at 4°C over 2 weeks. The concentration at each time point was measured in triplicates.

Comparison of the Cytometric Method and the ELISA Method

sFcεRIα in 48 serum samples from SLE patients and 32 serum samples from healthy controls were measured by both ELISA and our method for validation. The absolute concentrations of the samples were calculated according to the standard curve. The cutoff value of the two methods was obtained by three times the standard deviations above the mean level determined among the 32 healthy serum samples. In the 48 SLE samples, signal-to-cutoff value ≥ 1 was considered positive detection. Otherwise it was considered negative detection. In Figure 4, a comparison of the cytometric bead assay and ELISA is plotted to show correlations. Most QDs-encoded microbeads positive specimens were also positive by ELISA, and vice versa. In SLE samples, the two methods were correlated as shown in Supporting Information Figure S2 (Pearson coefficient = 0.213, $P = 0.037$). The standard curve of ELISA is shown in the Supporting Information Figure S3.

Correlation of sFcεRIα and IgE in SLE Patients and Healthy Volunteers

Quantification of sFcεRIα was determined in 149 serum samples in total (89 SLE serum samples and 60 healthy controls) with the newly developed method. Serum IgE was determined by clinical Siemens ADVIA 2400 diagnostic method. Table 7 shows the concentration of serum sFcεRIα and IgE in two groups. The serum sFcεRIα and IgE concentration

Table 6. Sample stability (mean $\pm$ SD)

RSFcεRI (ng/mL)	THREE FREEZE/THAW CYCLES (N = 3)	SIX FREEZE/THAW CYCLES (N = 3)	4°C OVERNIGHT (N = 3)
5	105.93% $\pm$ 2.19%	114.05% $\pm$ 3.76%	105.9% $\pm$ 6.16%
10	90.34% $\pm$ 3.51%	99.12% $\pm$ 13.08%	84.92% $\pm$ 10.14%
25	87.15% $\pm$ 5.88%	92.24% $\pm$ 6.84%	90.16% $\pm$ 11.7%
50	104.16% $\pm$ 4.13%	80.89% $\pm$ 2.82%	96.84% $\pm$ 5.16%
100	86.28% $\pm$ 4.52%	104.21% $\pm$ 3.43%	77.06% $\pm$ 11.07%
200	91.11% $\pm$ 4.69%	76.01% $\pm$ 21.9%	70% $\pm$ 2.96%

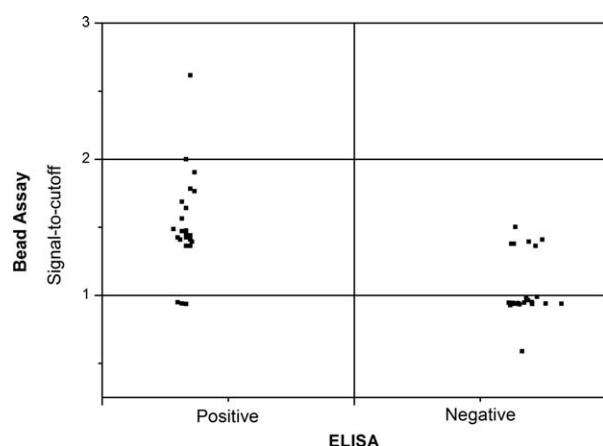


Figure 4. Signal-to-cutoff values of sFcεRIα levels relative to ELISA method.

differed between SLE patients and healthy controls ($P < 0.05$). SLE patients had significant higher level of sFcεRIα and IgE.

Table 8 shows the correlation analysis of sFcεRIα and IgE in two groups. For SLE patients and healthy controls, the concentration of serum FcεRIα and IgE had no correlation ($P > 0.05$).

DISCUSSION

We report here the establishment of a simple and quick quantitative assay for sFcεRIα using magnetic CuInS₂/ZnS quantum dots-encoded microbead-based flow cytometric technology. Our validation experiments demonstrated a good reproducibility and reliability of this novel method for the detection and quantification of sFcεRIα. Our method not only possessed better linearity and wider detection range, but also was more sensitive and convenient.

Development of a standardized quantification method that allows for the comparative analysis of serum sFcεRIα will facilitate the understanding of the clinical relevance of sFcεRIα in health and disease. In our study, NIR-emitting CuInS₂/ZnS QDs with superparamagnetic Fe₃O₄ nanoparticles in PSMA microbeads were successfully utilized to establish the cytometric microbead assay for sFcεRIα. The cadmium-free materials alleviated the toxicity of quantum dots (25). The addition of Fe₃O₄ nanoparticles eliminated the tedious washing steps by filtration, which made the method more convenient and efficient (21). The assay exhibited good linearity ($R^2 > 0.99$), and the LoD was established at 0.29 ng/mL with the dynamic range up to 200 ng/mL. The precision of the assay measured by intra- and inter-assay variability met the acceptance criteria for immunoassay validation with the mean recovery falling within 80–110% of the absolute concentration and a corresponding CV < 20%.

Table 8. Correlation analysis of sFcεRIα and IgE

	KENDALL'S TAU-B COEFFICIENT	P VALUE
SLE patients	−0.134	0.084
Healthy controls	0.035	0.695

In 2011, a study developed the ELISA method for sFcεRIα detection (26). Compared to ELISA, our method provide a more sensitive (LoD was 0.29 ng/mL) and high-throughput testing platform. Since there is no other reference for sFcεRIα quantification, we compared our test results with the commercial ELISA kit from the Affymetrix company. Serum sFcεRIα levels determined by the two methods yielded comparable results (Fig. 4), confirming our cytometric method as an acceptable method for the quantification of sFcεRIα. As depicted in Figure 4, minor inconsistencies in the two methods may be attributed to the usage of different standards and different affinity of antibodies. In addition, our methodology was measuring total soluble receptors. The coating antibody on the beads can capture both free sFcεRIα and preformed sFcεRIα-IgE complexes. Adding excessive IgE during the experimental process is to make sure that all sFcεRIα are bound with IgE and can be tested by PE conjugated anti-human IgE antibody. ELISA is a highly sensitive method. However, the enzymes are very susceptible to external environment and matrix constituents (27). In addition, the substrate color reaction is in a time-dependent manner, so strict compliance with the test protocol is mandatory. Opposite to enzymes, our method is less sensitive to environmental conditions (28).

SLE is a multisystem autoimmune disease that can potentially lead to widespread and severe organ damage or even death. It is characterized by autoantibodies to nuclear antigens, particularly double-stranded DNA (dsDNA). Diagnosis is primarily based on clinical symptoms and remains challenging because of the heterogeneity of the disease (29). Discovering more biomarkers or autoantibodies will help to distinguish SLE patients more safely and effectively. Recently, the presence of IgE autoantibodies binding to dsDNA has been reported in SLE patients and growing evidence suggests the pathogenic role of IgE in SLE (29,30). Increased serum IgE levels were observed in SLE patients in our experiment ($P < 0.05$) as displayed in Fig. 5. Studies have shown that the activation of basophils and the level of dsDNA-IgE antibody are correlated with the disease activity in SLE patients. By binding IgE to FcεRI, the mast cells or basophils are rapidly activated to release preformed cytokines, chemokines, and bradykinins that mediate manifestations of allergy. For the first time, we found that the serum sFcεRIα level was significantly higher in SLE patients than in controls ($P < 0.05$), which may be generated after FcεRI crosslinking-induced

Table 7. Quantification of serum sFcεRIα and IgE

	SLE PATIENTS (N = 89)	HEALTHY CONTROLS (N = 60)	P VALUE
sFcεRIα (ng/mL)	3.0241 (2.8024, 3.2666)	2.8091 (2.7226, 2.8503)	<0.001
IgE (IU/mL)	42.70 (12.20,153.1750)	15.90 (6.25,48.9750)	0.004

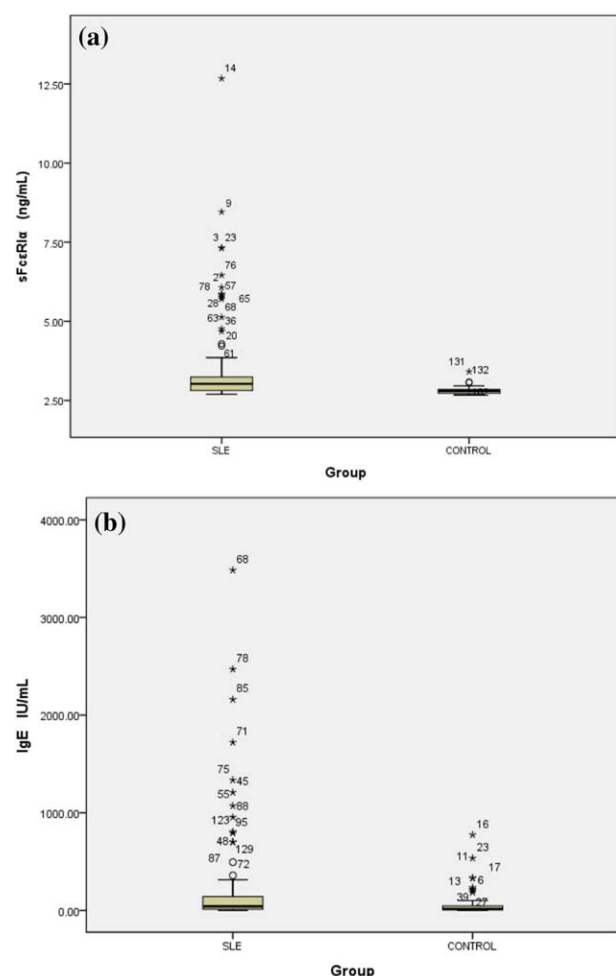


Figure 5. (a) Concentration of sFcεRIα in SLE patients and controls. (b) Concentration of IgE in SLE patients and controls. [Color figure can be viewed at wileyonlinelibrary.com]

receptor activation. Thus, a better understanding of sFcεRIα levels in health and disease will shed new light on the diagnostic strategy of SLE and other IgE-mediated immune diseases. Clinical laboratories are generally equipped with flow cytometry or Luminex for autoantibodies panel screening. This magnetic CuInS₂/ZnS quantum dots-encoded microbead-based flow cytometric technology can be implemented routinely with cytometry in the laboratories.

In summary, our work describes and validates a novel magnetic CuInS₂/ZnS quantum dots-encoded microbead-based flow cytometric method for quantification of sFcεRIα. Our assay shows good performance when dealing with serum samples. It is the first step toward a better understanding of the physiological role of sFcεRIα. Due to the low level of sFcεRIα in serum, complete elimination of serum matrix effect is still required to further improve the sensitivity of the assay. Furthermore, larger patient cohorts with multiple diseases are needed to explore whether sFcεRIα can be a biomarker for autoimmune or allergic diseases.

LITERATURE CITED

- Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365–378.
- Kinet JP. The high-affinity IgE receptor (FcεRI): From physiology to pathology. *Ann Rev Immunol* 1999;17:931–972.
- Wurzberg BA, Jardetzky TS. Structural insights into the interactions between human IgE and its high affinity receptor FcεRI. *Mol Immunol* 2002;38:1063–1072.
- Daeron M. Fc receptor biology. *Ann Rev Immunol* 1997;15:203–234.
- Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. *Nat Rev Immunol* 2014;14:247–259.
- Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: Data from the DEAL-HF study. *Clin Res Cardiol Off J German Cardiac Soc* 2010;99:323–328.
- Dehlink E, Platzter B, Baker AH, Larosa J, Pardo M, Dwyer P, Yen EH, Szépfalusi Z, Nurko S, Fiebigler E. A soluble form of the high affinity IgE receptor, Fc-εRI-RI, circulates in human serum. *PLoS One* 2011;6:e19098.
- Liu J, Ruppel J, Shire SJ. Interaction of human IgE with soluble forms of IgE high affinity receptors. *Pharmaceut Res* 1997;14:1388–1393.
- Naito K, Hirama M, Okumura K, Ra C. Recombinant soluble form of the human high-affinity receptor for IgE prevents anaphylactic shock in mice. *J Allerg Clin Immunol* 1996;97:773–780.
- Seminario MC, Saini SS, MacGlashan DW, Jr., Bochner BS. Intracellular expression and release of FcεRI α by human eosinophils. *J Immunol* 1999;162:6893–6900.
- Ra C, Kuromitsu S, Hirose T, Yasuda S, Furuichi K, Okumura K. Soluble human high-affinity receptor for IgE abrogates the IgE-mediated allergic reaction. *Int Immunol* 1993;5:47–54.
- Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW Jr. The relationship between serum IgE and surface levels of FcεRI on human leukocytes in various diseases: Correlation of expression with FcεRI on basophils but not on monocytes or eosinophils. *J Allerg Clin Immunol* 2000;106:514–520.
- Platzter B, Ruiter F, van der Mee J, Fiebigler E. Soluble IgE receptors—elements of the IgE network. *Immunol Lett* 2011;141:36–44.
- Messingham KA, Holahan HM, Fairley JA. Unraveling the significance of IgE autoantibodies in organ-specific autoimmunity: Lessons learned from bullous pemphigoid. *Immunol Res* 2014;59:273–278.
- Henault J, Riggs JM, Karnell JL, Liarski VM, Li J, Shirinian L, Xu L, Casey KA, Smith MA, Khatry DB, et al. Self-reactive IgE exacerbates interferon responses associated with autoimmunity. *Nat Immunol* 2016;17:196–203.
- Sabroe RA, Fiebigler E, Francis DM, Maurer D, Seed PT, Grattan CE, Black AK, Stingl G, Greaves MW, Barr RM. Classification of anti-FcεRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allerg Clin Immunol* 2002;110:492–499.
- Bossi F, Frossi B, Radillo O, Cugno M, Tedeschi A, Riboldi P, Asero R, Tedesco F, Pucillo C. Mast cells are critically involved in serum-mediated vascular leakage in chronic urticaria beyond high-affinity IgE receptor stimulation. *Allergy* 2011;66:1538–1545.
- Kim J, Biondi MJ, Feld JJ, Chan WC. Clinical validation of quantum dot barcode diagnostic technology. *ACS Nano* 2016;10:4742–4753.
- Tan EM, Cohen AS, Fries JE, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheumat* 1982;25:1271–1277.
- Liang L, Jean Daou T, Texier I, Chi TTK, Nguyen Quang Liem and Reiss P: Highly luminescent CuInS₂/ZnS core/shell nanocrystals: Cadmium-free quantum dots for in vivo imaging. *Chem Mater* 2009;21:2422–2429.
- Ding K, Jing L, Liu C, Hou Y, Gao M. Magnetically engineered Cd-free quantum dots as dual-modality probes for fluorescence/magnetic resonance imaging of tumors. *Biomaterials* 2014;35:1608–1617.
- Leng Y, Wu W, Li L, Kun L, Sun K, Chen X, Li W. Magnetic/fluorescent barcodes based on cadmium-free near-infrared-emitting quantum dots for multiplexed detection. *Adv Funct Mater* 2016;26:7581–7589.
- Wang X, Wang G, Li W, Zhao B, Xing B, Leng Y, Dou H, Sun K, Shen L, Yuan X, et al. NIR-emitting quantum dot-encoded microbeads through membrane emulsification for multiplexed immunoassays. *Small* 2013;9:3327–3335.
- Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev Aust Assoc Clin Biochem* 2008;29(Suppl 1):S49–S52.
- Pan Z, Mora-Sero I, Shen Q, Zhang H, Li Y, Zhao K, Wang J, Zhong X, Bisquert J. High-efficiency “green” quantum dot solar cells. *J Am Chem Soc* 2014;136:9203–9210.
- Lexmond W, der Mee J, Ruiter F, Platzter B, Stary G, Yen EH, Dehlink E, Nurko S, Fiebigler E. Development and validation of a standardized ELISA for the detection of soluble Fc-εRI-RI in human serum. *J Immunol Method* 2011;373:192–199.
- Speranskaya ES, Beloglazova NV, Abe S, Aubert T, Smet PF, Poelman D, Goryacheva IV, De Saeger S, Hens Z. Hydrophilic, bright CuInS₂ quantum dots as Cd-free fluorescent labels in quantitative immunoassay. *Langmuir ACS J Surf Colloid* 2014;30:7567–7575.
- Leng Y, Sun K, Chen X, Li W. Suspension arrays based on nanoparticle-encoded microspheres for high-throughput multiplexed detection. *Chem Soc Rev* 2015;44:5552–5595.
- Tsokos GC, Lo MS, Reis PC, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016;12:716–730.
- Bayry J. Lupus pathogenesis: Role of IgE autoantibodies. *Cell Res* 2016;26:271–272.

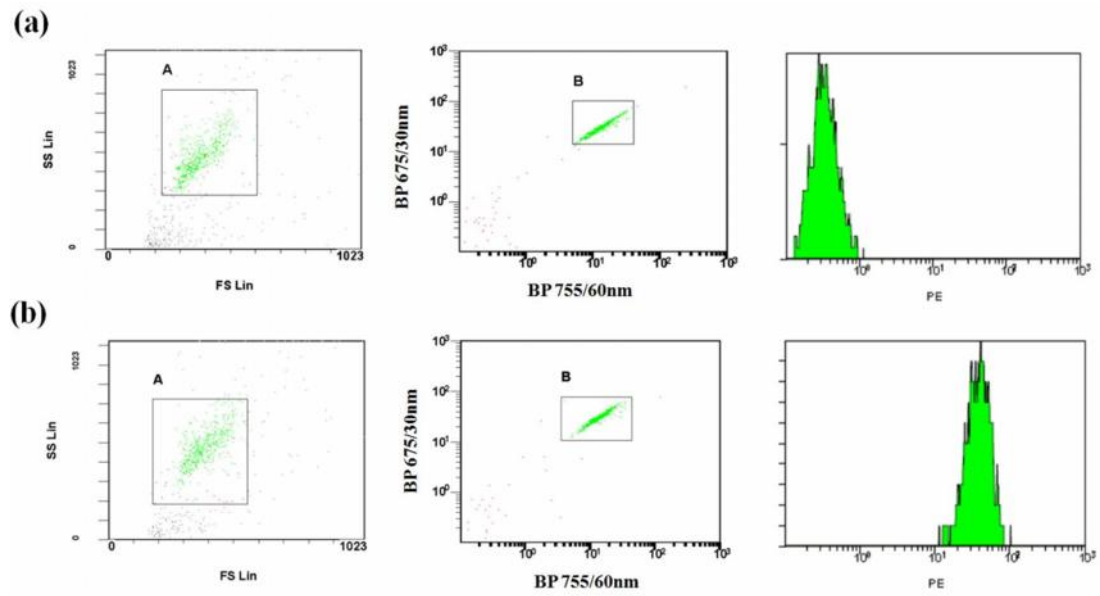


Figure S1. An example of the testing data. (a) The cytometric plot of a healthy volunteer; (b) The cytometric plot of a SLE patient.

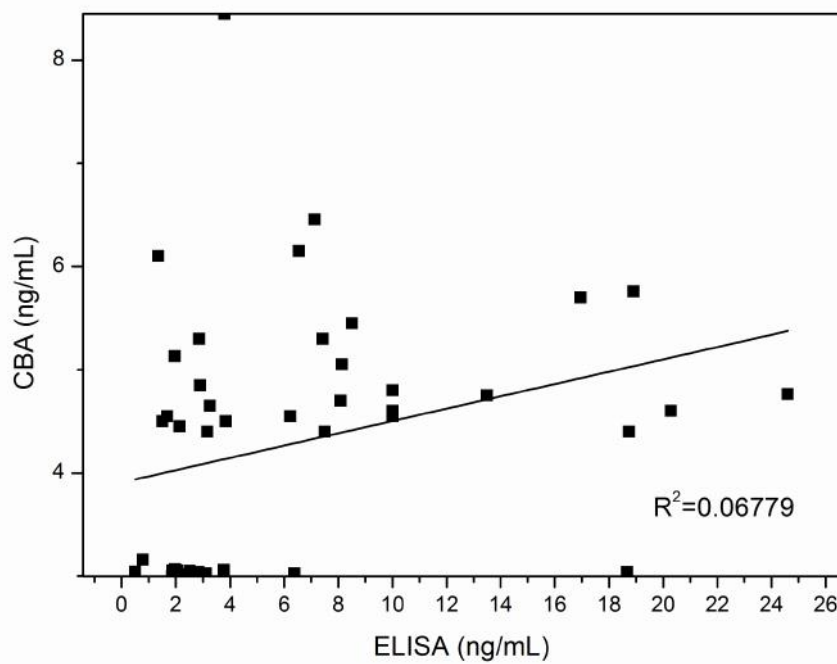


Figure S2. Correlations of concentrations obtained from patient serum samples by using bead based assay and ELISA method.

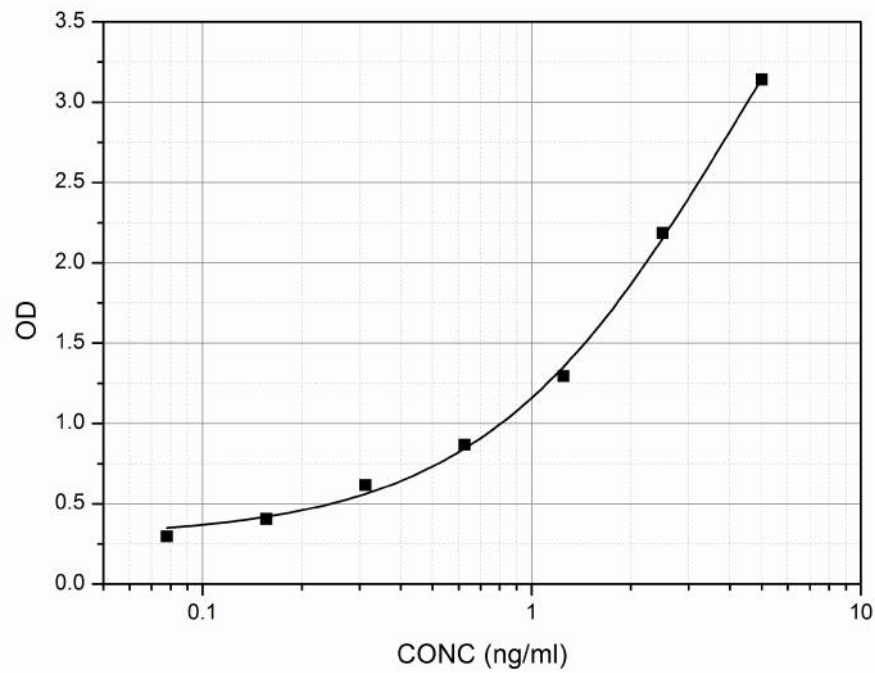


Figure S3. The standard curve of ELISA method. The detection range is from 0.078 ng/mL to 5 ng/mL following the manufacture's instruction.

PE conjugated anti-human IgE

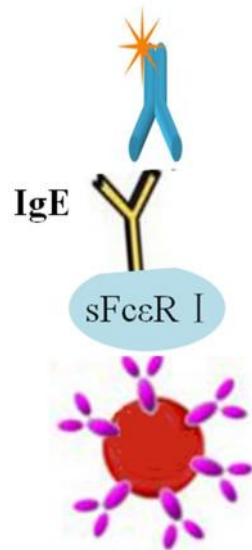


Figure S4. The schematic illustration of the assay procedure.