



Development of a light-initiated chemiluminescent assay for the quantitation of sIgE against egg white allergens based on component-resolved diagnosis

Ying Bian¹ · Changshan Liu² · Tiantian She³ · Mengjuan Wang² · Juanjuan Yan³ · Dianjun Wei¹ · Huiqiang Li³

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Abstract

The determination of specific IgE (sIgE) level is of great importance in IgE-mediated food allergies. Our aim was to develop a homogeneous immunoassay—light-initiated chemiluminescent assay (LICA)—for measuring allergen sIgE of a single component in egg white, thus evaluating the LICA-sIgE assay as a useful tool in the diagnosis of food allergy. The LICA-sIgE assay was performed by incubating serum sample with anti-human IgE antibody coated with chemiluminescer beads, streptavidin-coated sensitizer beads, and biotinylated antigens, which consist of four components in egg white. Serum samples from egg allergic patients ($n = 70$) and healthy volunteers ($n = 30$) were collected. For calibration, purified human IgE was used as the calibrator. Working conditions of this homogeneous immunoassay were optimized, analytical performance was determined, and correlation of the results between LICA and ImmunoCAP was evaluated. The assays were performed in 8-well plates with a sample volume diluted to 1:10 of 25 μ l. Intra-assay precision (% coefficient of variation) ranged from 1.83 to 4.13%, and inter-assay precision ranged from 2.70 to 8.70%. It exhibited excellent sensitivity, which could distinguish between positive samples and negative samples even at a large dilution level. The sIgE-LICA and ImmunoCAP correlated well in patients allergic to single component ($r^2 = 0.929$). Also, the components ovomucoid and ovalbumin were best at predicting ImmunoCAP results, with the same area under the ROC curve (AUC) of 0.81, and a specificity of 90.0 and 93.3%, respectively. Our data show effective performance characteristics of LICA to detect sIgE in human serum based on component-resolved diagnostic tests (CRD). The homogeneous sIgE-LICA assay has the following key advantages: requires no washing, simplicity and rapidity, reproducibility, high-throughput, good performance in a liquid phase assay, and good suitability for sIgE diagnosis in food allergy based on CRD.

Keywords LICA · sIgE · Egg white · CRD

Introduction

The specific IgE (sIgE) assay has become one of the most important diagnostic tools in IgE-mediated food allergy

[1–4]. The quantitative measurement of serum sIgE level is helpful in determining the predisposition of food allergy resolution for patients. At present, several in vitro laboratory tests for measuring sIgE are available. Since IgE was discovered in 1967 [5], the radioallergosorbent test (RAST) had been the first routine technique for sIgE detection in serum. Subsequent second-generation test systems like the fluorescence enzyme immunoassay (FEIA) have evolved and have been used widely. These methods have the advantages of greater speed, higher binding capacity, and use of nonisotopic labels, but they are also limited by sample handling, turnaround time, laboratory integration, and most importantly, solid-phase allergen immobilization [6, 7]. Pharmacia ImmunoCAP technology, which is a well-established in vitro test for the determination of sIgE, is

✉ Huiqiang Li
lhq@tmu.edu.cn

¹ Department of Clinical Laboratory, Second Hospital of Tianjin Medical University, 23 Pingjiang Road Hexi District, Tianjin 300211, China

² Department of Pediatrics, Second Hospital of Tianjin Medical University, Tianjin 300211, China

³ Department of Clinical Laboratory, School of Medical Laboratory, Tianjin Medical University, Tianjin 300203, China

regarded as the quasi-standard to which other methods are compared [8]. However, it also suffers from the limitations mentioned above [9].

The accurate quantitation of sIgE level mainly depends on the difference in proteins. Traditional *in vitro* sIgE tests are based on crude extracts composed of allergenic and non-allergenic molecules. Such samples cannot provide information about which allergen component in the extracts is specifically recognized by the sIgE and the presence of cross-reactivity also affect sIgE results [10, 11]. Allergen component-resolved diagnostic test (CRD) is a new method in food allergy diagnosis. It uses purified or recombinant allergens at the molecular level to identify sensitization of a patient [12]. CRD effectively distinguishes genuine sensitization from sensitization due to cross-reactivity, and it provides the IgE profile of a broad panel of allergens to which the specific patient is sensitive [10, 13–15].

Here we introduce a third-generation sIgE test system based on a light-initiated chemiluminescent assay (LICA). Just like proximity-based AlphaLISA® and AlphaScreen®, which have been developed to identify the interaction between small molecules such as protein-protein interactions [16, 17]. LICA is a bead-based immunoassay. The sensitizer beads and chemibeads are brought into close proximity (< 200 nm) when analyte molecules are captured, resulting in the emission of light by chemiluminescence [18–20]. The principle of this methodology is shown in Fig. 1a. It is a homogeneous non-radioactive immunoassay which is rapid, sensitive, reproducible, requires no washing, and is effective for high throughput detection of sIgE [21]. In this study, we examined the IgE profile of an egg white allergens panel using LICA. Four components were identified as major allergens in egg white, including ovomucoid (OM; Gal d 1), ovalbumin (OA; Gal d 2), ovotransferrin (OT; Gal d 3), and lysozyme (Gal d 4) [22].

We optimized working conditions in the LICA-sIgE assay, evaluated its analytical performances, and compared the results obtained by LICA with results from the ImmunoCAP technology. Based on CRD, we then assessed the ability of a single component in egg white to predict egg allergy.

Materials and methods

Samples

Sera samples from 70 egg allergic patients were obtained from the Second Hospital of Tianjin Medical University. Allergy to egg was determined by a convincing clinical history of symptoms (urticaria, respiratory symptoms, or gastrointestinal symptoms), by the presence of serum total IgE (> 100 kU_A/L), and by an egg white-specific IgE level of at least 0.35 kU_A/L, analyzed with the ImmunoCAP System (Thermo Fisher Scientific, Sweden). Sera from 30 healthy volunteers (no history of allergic symptoms, negative serum egg white sIgE level) were collected as negative controls. All sera samples were stored in aliquots at –20 °C. Informed consent was obtained from patients and healthy individuals. The study was approved by the Ethics Committee of the Tianjin Medical University.

Apparatus and chemicals

The sensitizer beads coated with streptavidin and the chemibeads were obtained from Beyond Biotech (Shanghai, China). Four major egg white allergen proteins (Gal d 1, Gal d 2, Gal d 3, Gal d 4) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Human IgE used as calibrators was from Abbotec (San Diego, CA, USA), and the purity

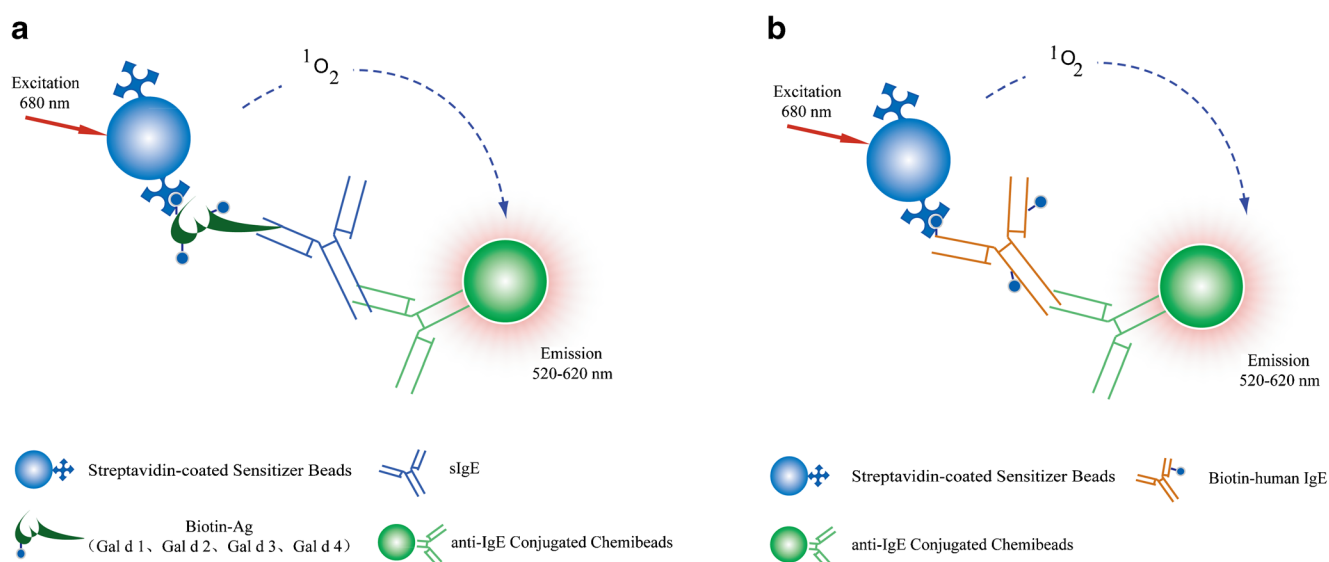


Fig. 1 **a** Principle of LICA for measuring allergen-sIgE in egg white. **b** Principle of calibration in LICA for measuring IgE

was >98% by SDS-PAGE analysis. The monoclonal anti-human IgE antibody was purchased from Abcam (Cambridge, UK). The Sulfo-NHS-LC-Biotin was purchased from Pierce (Rockford, IL). The assays were performed in 8-well plates (Beyond Biotech, China), and the high throughput chemiluminescence analyzer instrument used to carry out the LICA tests was from Beyond Biotech (Shanghai, China).

LICA detection protocol

Biotinylation of antigens

The four major allergen proteins of egg white were biotinylated by Sulfo-NHS-LC-Biotin. The antigens were dialyzed in carbonate buffer solution (pH 8.0) and incubated overnight at 4 °C with stirring. Dropwise, Sulfo-NHS-LC-Biotin in 0.1 M phosphate buffer were added to achieve a molar ratio of 20:1, and kept for 1 h at room temperature. To quench the reaction and remove the unconjugated biotin reagent, the mixture was then dialyzed with 0.1 M phosphate buffer at 4 °C for 24 h. The resulting product was stored at 4 °C.

Coupling of antibody to beads

The monoclonal anti-human IgE antibody was coated onto the chemibeads according to standard procedures as described [20]. Chemibeads was pretreated in 0.1 M phosphate buffer (pH 7.0) by sonication, and 0.1 mg of antibody was dialyzed in 50 mM 2(*N*-morpholino) ethanesulfonic acid (MES) buffer (pH 6.0) to obtain an antibody solution. The antibody solution and 2 mg of acceptor beads were mixed quickly. Then, the mixture was added to sodium cyanotrihydridoborate (NaCNBH₃) at a volume ratio of 25:1, and incubated at 37 °C for 48 h. After this incubation, the resulting product was washed with MES by centrifugation. Immunoluminescent chemibeads were resuspended at 10 mg/ml and stored at 4 °C.

Assay procedure

The assay was conducted in 0.1 M Tris·HCl with 3% bovine serum albumin (BSA), and was adjusted to pH 8.0. The assay procedures were performed in two simple steps. Briefly, diluted samples of serum or calibrators (25 µl) were added into the 8-well plates, then 25 µl of biotinylated antigen and 25 µl of chemibeads coated by anti-human IgE antibody were added. The plates were placed in the chemiluminescence analyzer instrument for agitation and incubation at 37 °C for 30 min, which ensured sufficient mixing of the reagents and promoted the speed of reaction. After that, LICA Common Reagent (150 µl), which contains streptavidin-coated sensitizer beads, was added under the green light. The plates were placed back in the instrument, and the reaction was incubated at 37 °C for another 10 min in the dark. Then, the results were read on the

LICA instrument. Samples were performed in duplicate in the plates. The total measuring time of the LICA immunoassay was 40 min.

Calibration

We used purified human IgE as a positive calibrator [23]. The samples of purified human IgE were biotinylated by Sulfo-NHS-LC-Biotin as mentioned above. The schematic diagram is shown in Fig. 1b. For calibration, solutions of biotin-conjugated human IgE were serially diluted to 0.435, 0.217, 0.109, 0.054, 0.013 IU/ml, and human serum albumin (HSA) was used as the negative control and evaluated as 0 IU/ml. The intensity of the chemiluminescence (CL) signal increased linearly with the rise of biotin-conjugated human IgE concentration. We then extrapolated the level of specific IgE from the dose-response curve of total IgE [24]. Sera from patients (eight cases) who were allergic to a single allergen protein in egg white were selected, and the concentrations of sIgE were analyzed by ImmunoCAP System and were recorded. Then, the results of sIgE concentration according to the dose-response curve of total IgE were adjusted to the results of ImmunoCAP System, thus obtaining a dose-response curve of sIgE (Fig. 2). Each concentration was analyzed in duplicate. The calibrator solutions were stored at 4 °C, and the eight serum samples were stored in aliquots at −20 °C.

Optimization of the reaction conditions

The optimal LICA working conditions were affected by a number of factors. To develop a highly effective homogeneous chemiluminescence immunoassay method for the detection of sIgE, we optimized biotinylated antigens, serum dilution, the concentration of chemibeads, the concentration of anti-human IgE antibody, and the incubation time of the

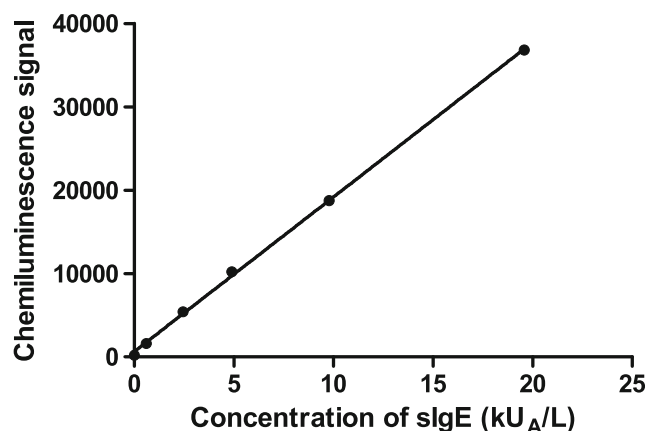


Fig. 2 Calibration curve of the light-initiated chemiluminescent assay (LICA) for detecting sIgE in human sera. Each point represents the mean value of replicate measurements ($n = 2$). Error bars represent standard deviations. Where the error bars are invisible, the magnitudes of them are smaller than the point symbols

first step. In this study, we used a major allergen in egg white, ovomucoid (Gal d 1), to optimize the working conditions. Sera pools containing high levels of sIgE and low levels of sIgE were collected, and the concentrations of the two sera pools were about 2.0 and 0.5 kU_A/L, respectively. Sera from nonallergic individuals served as negative controls. The positive sera pool and negative sera pool were designated as P and N, respectively. Positive to negative (P/N) ratios were calculated.

Assay performance characteristics of sIgE measurement by LICA

Precision

Three sera pools containing high, medium, and low levels of sIgE for each component in egg white were collected. Samples were stored as single ready-to-use aliquots at -20°C . For the intra-assay study, each sample was measured 20 times in one analytical run. For the inter-assay study, each sample was analyzed in the same manner for 20 consecutive days, one duplicate per day. To determine the intra-assay variation and inter-assay variation, average measured value (kU_A/L), SD, and coefficients of variation (CV) were calculated.

Sensitivity

To investigate the sensitivity of the sIgE assay, three sera pools containing high, medium, and low levels of sIgE were collected as described above. The concentrations of the three sera pools were about 3.8, 0.7, and 0.1 kU_A/L, respectively. Each sample was serially diluted in assay buffer. After several times of dilution, the difference of the chemiluminescence signal (CL signal) between the diluted positive sample and the negative sample was observed.

Accuracy

To validate the analytical recovery of this assay, four serum samples containing sIgE concentration of the corresponding component from 2.90 to 13.40 kU_A/L, respectively, were diluted with the IgE-depleted sera pool. All these samples were measured according to the assay procedure of LICA. The recovery rate was determined as (measured concentration) / (expected concentration) $\times 100\%$.

Receiver operating characteristic curves for LICA as screening tests to egg allergy ($n = 100$)

All 70 egg allergic patients and 30 healthy volunteers participating in this study underwent screening tests by LICA to egg components Gal d 1, 2, 3, and 4. Diagnostic performance of a

single component was measured by constructing receiver operating characteristic (ROC) curves, with the extract-based ImmunoCAP results as reference. To evaluate the clinical usefulness, i.e., the test's performance in distinguishing between patients with and without egg allergy, sensitivity, specificity, area under the curve (AUC) were calculated, and the optimal cut-off levels which could simultaneously maximize sensitivity and specificity to predict egg allergy were selected.

Results

Calibration curve

A representative calibration curve for sIgE in human sera is shown in Fig. 2. Using the biotin-conjugated purified human IgE as a calibrator, six concentrations including the zero calibrator were selected to build a dose-response curve of total IgE. Then, the sIgE values of patients allergic to a single allergen in this curve were adjusted to the results of ImmunoCAP System to generate a dose-response curve of sIgE ($y = 636.05 + 1854.33 \times x$). The final standard curve presented an intensity range between 180 and 40,000 counts per second, which corresponded to a sIgE concentration range of 0–20 kU_A/L. The correlation coefficient of $r = 0.999$ indicated an excellent linear quantification.

Optimization of serum dilution and the concentration of biotinylated antigens

We observed an optimal reacting concentration between antigen and sIgE in human sera. Figure 3a revealed that a concentration of 0.665 $\mu\text{g/ml}$ biotinylated antigen was sufficient to bind with sIgE. The CL signal increased with increasing serum dilution titration in the range 1:40–1:10 (Fig. 3b). Because the proportion of other irrelevant proteins in human sera could affect the assay properties, we selected the dilution of serum to 1:10 by maximal effective multiple to eliminate the interference. Therefore, a serum dilution titration of 1:10 and a concentration of 0.665 $\mu\text{g/ml}$ for the biotinylated antigen were used in subsequent experiments.

Optimization of chemibeads and anti-IgE antibody concentration

The concentration of chemibeads and anti-IgE antibody were important factors for the immunoassay sensitivity and linear range. Excess amount of chemibeads would provide more chances for random collisions between the sensitizer beads and chemibeads, which subsequently increased the background signal and decreased the linear range. Also, too few of the chemibeads would decrease the CL signal and affect the immunoassay sensitivity. In this assay, the anti-IgE antibody

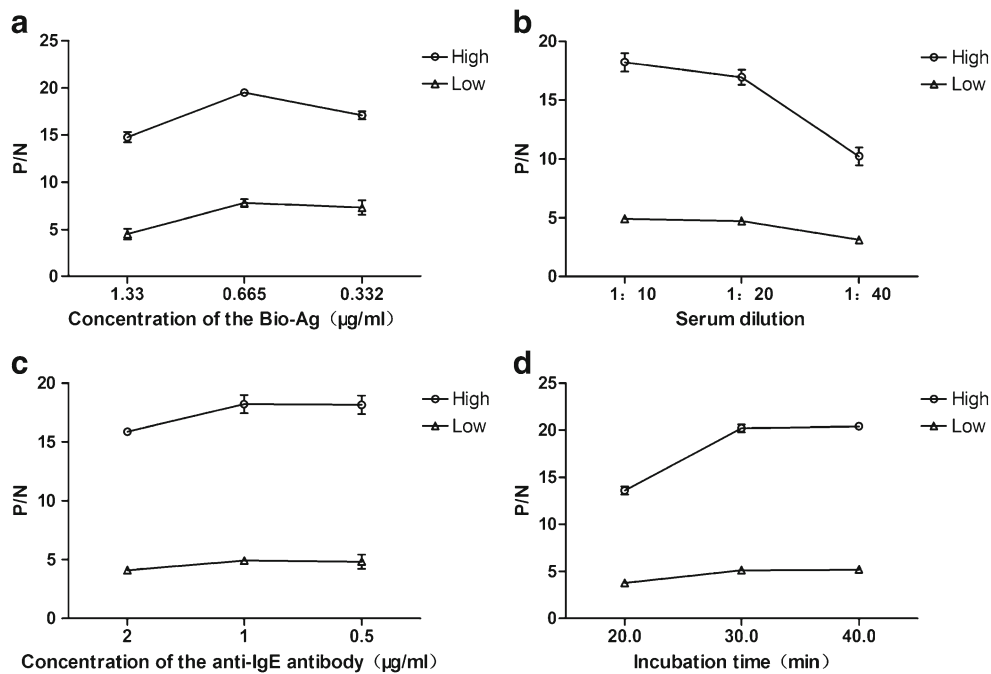


Fig. 3 Optimization of LICA-sIgE reacting conditions. **a** The effect of different concentrations of biotinylated antigens, keeping the serum dilution and anti-IgE antibody concentration and incubation time invariable. **b** The effect of different serum dilution titrations, keeping the anti-IgE antibody and biotinylated antigen concentration and incubation time invariable. **c** The effect of different concentrations of

anti-IgE antibody, the serum dilution titration, biotinylated antigen concentration, and incubation time were invariable. **d** The effect of different incubation time in the first step, keeping other variables constant. P/N represents positive to negative (P/N) ratios, the two lines represent high levels and low levels of sIgE sera pool, respectively. Error bars indicate the range values of measurements

was serially diluted to 2, 1, and 0.5 $\mu\text{g/ml}$ in assay buffer, and accordingly the chemibeads concentrations were 40, 20, and 10 $\mu\text{g/ml}$. To ensure an excess of antibody incorporated in this assay, a concentration of anti-IgE antibody of 2 $\mu\text{g/ml}$ was superior to 0.5 $\mu\text{g/ml}$. But the P/N ratios decreased with increasing anti-IgE antibody concentration in the range 1–2 $\mu\text{g/ml}$ because too many chemibeads increased the background signal, as shown in Fig. 3c. Concentrations of 1 and 20 $\mu\text{g/ml}$ of anti-IgE antibody and chemibeads, respectively, were used in subsequent experiments.

Optimization of time used for incubation

The time required for the first incubation step was established by conducting this incubation for 20, 30, and 40 min, keeping the second incubation time constant (10 min). The CL signal increased with increasing incubation time over the range of 20–30 min (Fig. 3d). After 30 min, there was little further change in signal, indicating the reaction between antigen and antibody had reached an equilibrium status. Thus, we determined that 30 min was the optimal incubation time for the first step. The whole reaction was very fast and completed within 1 h for all IgE levels tested.

Assay precision

The assay precision of the four components in egg white (Gal d 1, 2, 3, 4) was shown in Table 1. For the intra-assay, coefficients of variation (CV) of the four components varied from 1.83 to 4.13%. For the inter-assay variability, CV values varied from 2.70 to 8.70%, with both being below 10.00%. Thus, the excellent reproducibility and precision of the LICA-sIgE assay could be attributed to the no-wash, highly-automated, and rapid assay conditions that avoided blocking and multiple washing steps, which might produce unpredictable signal variation.

Assay sensitivity

The assay sensitivity of the four components in egg white (Gal d 1, 2, 3, 4) was shown in Fig. 4. Three sera pools containing high, medium, and low levels of sIgE for each component were serially diluted. The high level of sIgE sera pool was diluted to 5120-fold, the medium level to 1280-fold, and the low level to 160-fold. Even though the sera were diluted to such a large scale, a difference of the CL signal between the diluted positive sample and the negative sample was evident, indicating good sensitivity of the LICA-sIgE assay.

Table 1 The intra-assay precision and inter-assay precision of the four components in egg white

Intra-assay (<i>n</i> = 20)			Inter-assay (<i>n</i> = 20, detection of 20 days)	
	Average measured value (kU _A /L)	CV (%)	Average measured value (kU _A /L)	CV (%)
a. Gal d 1				
High	4.42 ± 0.191	4.32	4.54 ± 0.257	5.66
Middle	1.53 ± 0.042	2.74	1.51 ± 0.069	4.57
Low	0.135 ± 0.0064	4.74	0.129 ± 0.0110	8.53
b. Gal d 2				
High	3.47 ± 0.056	1.61	3.35 ± 0.146	4.36
Middle	0.92 ± 0.044	4.78	0.98 ± 0.073	7.45
Low	0.131 ± 0.0087	6.64	0.130 ± 0.0099	7.61
c. Gal d 3				
High	5.61 ± 0.087	1.55	5.70 ± 0.193	3.38
Middle	0.98 ± 0.052	5.31	0.93 ± 0.079	8.49
Low	0.234 ± 0.0132	5.64	0.262 ± 0.0157	5.99
d. Gal d 4				
High	3.88 ± 0.081	2.09	3.79 ± 0.135	3.56
Middle	1.43 ± 0.058	4.05	1.47 ± 0.079	5.37
Low	0.138 ± 0.0081	5.87	0.142 ± 0.0105	7.39

Assay accuracy

The assay accuracy was evaluated by calculating the ratio between the measured and expected values, as shown in

Table 2. The recovery rate ranged from 90.29 to 117.93%. Most of the results performed complete parallelism, indicating that there was a linear relationship between the sample volume and assay response. The maximum deviation of recovery was

Fig. 4 Sensitivity of the LICA-sIgE assay. Discrepancy between the diluted positive sample and the negative sample could be observed even at a large dilution titration level. Error bars represent standard deviations. Where the error bars are invisible, the magnitudes of them are smaller than the point symbols

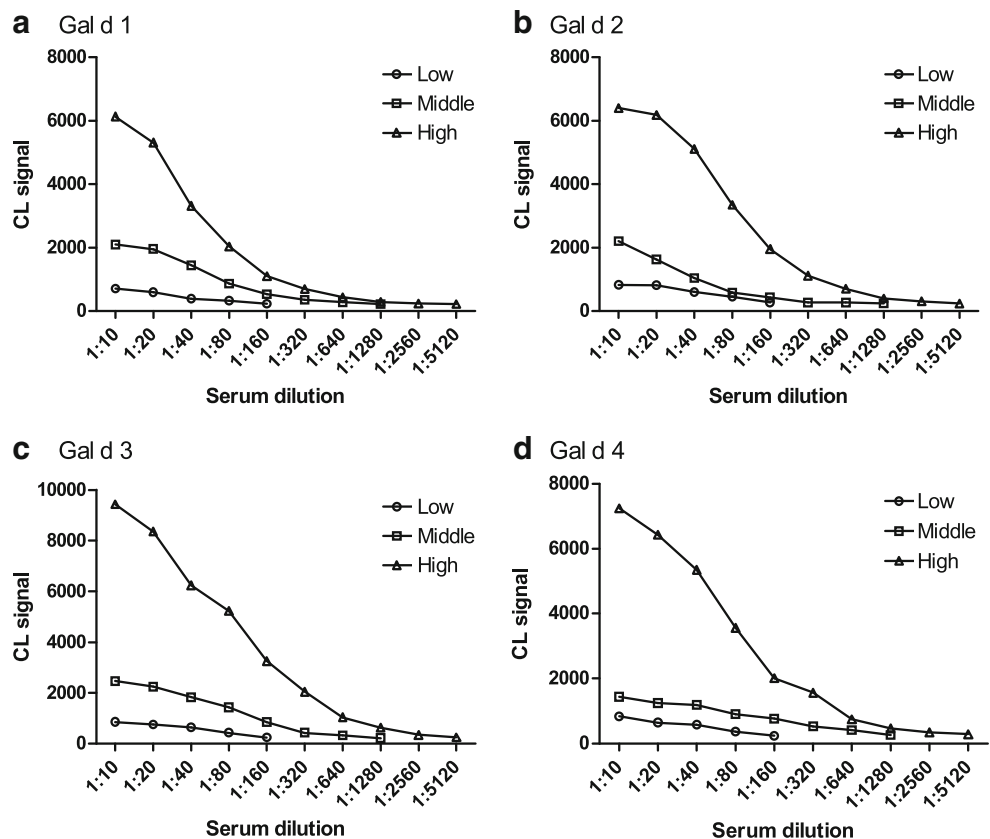


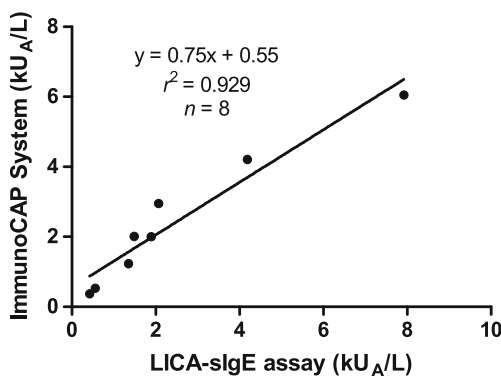
Table 2 Linearity of dilution of human serum samples

Component	Sample No.	Dilution	Measured sIgE (kU _A /L)	Expected sIgE (kU _A /L)	Recovery (%)
Gal d 1	1	Neat	4.12	4.12	100.00
		1:2	1.96	2.06	95.15
		1:4	0.93	1.03	90.29
		1:8	0.48	0.52	92.31
Gal d 2	2	Neat	13.40	13.40	100.00
		1:2	7.60	6.70	113.43
		1:4	3.66	3.35	109.25
		1:8	1.82	1.68	108.33
Gal d 3	3	Neat	3.21	3.21	100.00
		1:2	1.66	1.61	103.11
		1:4	0.85	0.80	106.25
		1:8	0.46	0.40	115.00
Gal d 4	4	Neat	2.90	2.90	100.00
		1:2	1.71	1.45	117.93
		1:4	0.76	0.73	104.11
		1:8	0.39	0.36	108.33

117.93%, which occurred when sample No. 4 was diluted as 1:2. This might be because when we were diluting, the sIgE concentration changed; also, the antibody valence changed as well, thus resulting in the slight difference between sample volume and assay response. In short, the accuracy of the LICA-sIgE assay that we developed was acceptable.

Comparison with ImmunoCAP

Of all the 70 egg allergic patients, there were only 8 people who were allergic to a single component in egg white. We analyzed sera from the 8 patients with sIgE concentrations ranging from 0.43 to 7.92 kU_A/L with the LICA-sIgE assay, and simultaneously determined with ImmunoCAP System. Regression analysis indicated good correlation between the two methods, as shown in Fig. 5. This result gave further evidence to confirm the accuracy of the LICA-sIgE assay.

**Fig. 5** Correlation between sIgE levels in serum samples measured by LICA-sIgE assay and ImmunoCAP System

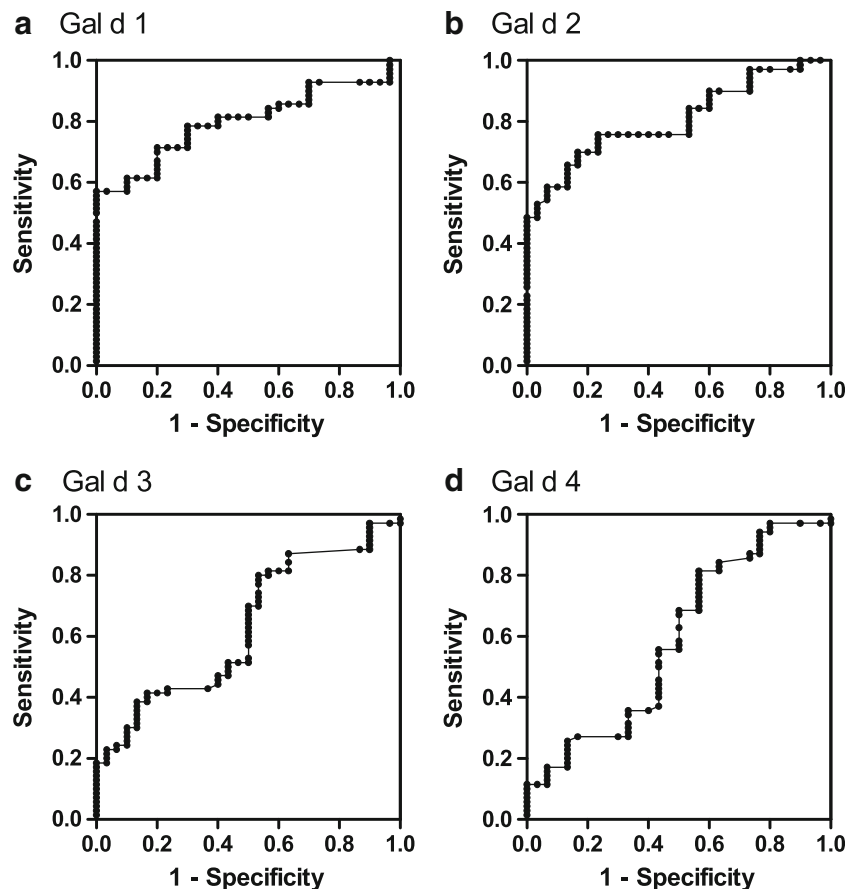
ROC analysis

In the ROC analysis of sIgE to the individual egg white components for predicting egg allergy, we used the method of LICA according to the allergen component-resolved diagnostic testing (CRD). The ROC curves for component Gal d 1 and Gal d 2 displayed identical and highest AUC values of 0.81, followed by the component Gal d 3 and Gal d 4 with AUC values of 0.63 and 0.59, respectively (Fig. 6). For component Gal d 1, we chose a cut-off value of ≥ 773 (CL signal) for our test, achieving the sensitivity of 61.4% and specificity of 90.0%. Similarly, component Gal d 2, with an optimal cut-off value ≥ 797 , achieved the sensitivity of 58.6% and specificity of 93.3%. Components Gal d 3 and Gal d 4 were much inferior with respect to the sensitivity and specificity at their optimal cut-off points. However, if the components were combined, such as Gal d 1 and Gal d 2, the sensitivity was greatly improved and reached 72.4%.

Discussion

In this study, we developed a homogeneous high-throughput immunoassay for quantitative detection of sIgE in human serum. Like the luminescent oxygen channeling immunoassay (LOCI), the ultimate principle of LICA is based on singlet oxygen transmission. The phthalocyanine served as the photosensitizer in the sensitizer beads and can activate ambient oxygen to singlet oxygen under irradiation at 680 nm. With sIgE recognition of antigens and anti-IgE antibody, bringing the sensitizer beads and chemibeads into close proximity, the singlet oxygen molecules can react with thioxene derivatives

Fig. 6 Receiver operating characteristic (ROC) curves for LICA tests with CRD in predicting egg allergy in overall population ($n = 100$)



in the chemibeads, culminating in light production at 520 to 620 nm. The LICA signal increases following the rise of sIgE concentration in human serum. However, the singlet oxygen is short-lived with a half-time of only 4 μ s and diffuses only a short distance (< 200 nm) [25]. Without specific interaction of the biomolecules, there can be no light signal.

For calibration, we used purified human IgE as the calibrator. A close relationship was observed between the sIgE concentrations of eight patients who were only allergic to a single component in egg white with their ImmunoCAP results ($r^2 = 0.929$). Such results suggested that an application of purified IgE for the quantitative measurement of sIgE was feasible. Discrepancies between LICA and ImmunoCAP could be due to diversity and quality of the allergens used in the two immunoassays [26, 27]. The antigen used in LICA was a single component conjugated with Sulfo-NHS-LC-Biotin in a liquid phase immunoassay, while egg white allergen extracts in ImmunoCAP were immobilized in a three-dimensional cellulose solid allergen phase [28]. Differences in allergenic epitopes which sIgE specifically recognized may account for this outcome. We believe a better correlation between the two immunoassays could be achieved if the allergens used were more similar.

The sIgE-LICA assay exhibited a range of important performance characteristics such as reproducibility, sensitivity,

sample volume, assay time, ease of handling, larger positive to negative (P/N) ratio, and a liquid phase reaction environment. The homogenous immunoassay required no washing steps, thus eliminating the need for blocking and multiple washing steps which are essential in many other assays like ELISA, and that often resulted in analyte dilution, human contamination, and unpredictable signal variation [20]. Also, the sIgE-LICA assay exhibited excellent sensitivity because of the amplified signal resulting from 60,000 singlet oxygen molecules generated by each sensitizer bead [29]. Furthermore, the simplicity of reagent generation was an important consideration. The antibody labeling of the chemibeads was a facile, efficient process that could be performed with standard laboratory equipment. The antibody-labeled chemibeads and streptavidin-coated sensitizer beads were common reagents, which removed the need for costly and time-consuming generation of custom reagents [21].

When we were using CRD to examine the four allergens in egg white based on sIgE-LICA, components Gal d 1 and Gal d 2 showed the same highest AUC of 0.81, which were superior in the prediction of egg allergy. The two components displayed good specificity (90.0 and 93.3%, respectively), but poor sensitivity (61.4 and 58.6%, respectively). The quantity and quality of the allergen which sIgE specifically recognized could account for this outcome. Patients who were

allergic to egg white might be allergic to multiple components of egg white and might only be allergic to one component. So, it was of great importance to establish an accurate patient sensitization profile. In addition, we speculate that ‘component-resolved immunotherapy’ could be a next step in the fine-tuning of allergen-specific immunotherapy by targeting only relevant allergens from a specific source [30].

As CRD has been gradually introduced into clinical food allergy diagnosis, investigations of CRD revealed that this method was able to predict the severity of allergic reactions and to distinguish between patients with symptomatic allergy and those who were sensitized, but clinically tolerant toward allergens such as apple, cherry, hazelnut, carrot, and, particularly, wheat [31, 32]. In egg white, the heat-stable protein Gal d 1 has been regarded as the dominant allergen. Studies showed that an elevated level of Gal d 1 sIgE was a negative prognostic marker in egg allergy, and it was associated with the persistence of egg allergy [33, 34]. Other studies have been conducted to evaluate the utility of Gal d 1 sIgE level in predicting tolerance to egg white. Research by Gray et al. reported that ImmunoCAP Gal d 1 was best at differentiating true egg allergy from tolerance, as compared with SPT to raw egg white and ImmunoCAP to egg white [35]. Also, Ando et al. found that in the diagnosis of tolerance to heated egg, the Gal d 1 sIgE level was slightly superior to egg white IgE level [33]. Overall, component Gal d 1 could be used as an adjunct to the currently used allergy tests to avoid performing some OFCs, but it is not presently ready to be used alone [36].

Accurate quantitation of sIgE is of critical importance in the diagnosis of food allergy. In the current article, we have described the establishment of LICA to detect sIgE in human serum based on CRD. This homogenous immunoassay exhibited excellent performance characteristics, and it was expected to be considered as an analytical tool in clinical practice.

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Compliance with ethical standards The authors declare that they have no conflicts of interest. This research involved sera from allergic patients and healthy individuals. Informed consent was obtained from all human participants.

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