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Egg white component-resolved diagnosis: Testing of serum ovalbumin-specific IgE by luminescent oxygen channeling immunoassay

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ABSTRACT

Egg allergy is the second most common food allergy in children. Precise measurement of allergen component-specific IgE (sIgE) in serum contributes to molecular diagnosis of egg allergy, i.e. component-resolved diagnosis (CRD). Existing CRD methods, including ImmunoCAP and Immulite, have unavoidable drawbacks such as errors from solid-liquid phase separation and enzyme instability. Therefore, we take egg allergen ovalbumin as an example to establish a homogenous method – indirect luminescent oxygen channeling immunoassay (LOCI) to determine ovalbumin-sIgE precisely. In the indirect LOCI reaction system, ovalbumin-conjugated acceptor nanoparticles were brought close to streptavidin-conjugated donor nanoparticles through antigen-antibody and biotin-streptavidin interactions. The optimal conditions for LOCI were 16.67 μg/mL acceptor-ovalbumin, 1.047 μg/mL biotinylated anti-human IgE antibodies and a 1/20 dilution of serum in a 25-μL reaction volume for a 45-min incubation. We assessed the performance of of this LOCI system, including sensitivity, precision, and anti-interference ability, and compared the diagnostic performance of the LOCI with enzyme-linked immunosorbent assay (ELISA) through a receiver operator characteristic (ROC) analysis. Results revealed that LOCI was superior to ELISA in performance. In summary, we demonstrated that indirect LOCI was an excellent method for precise determination of allergen component-sIgE, contributing to CRD of food allergy.

1. Introduction

Food allergy is a health problem with growing prevalence worldwide [1]. For IgE-mediated food allergies, in vitro testing of allergen-specific IgE (sIgE) in human serum remains an essential diagnostic tool [2]. Conventionally, the crude extract of an allergenic food is used as a known antigen to detect sIgE in serum. However, due to the complex composition of allergenic foods, some low abundance allergen components can be easily lost during the extraction process [3]. Moreover, the allergenic food sources and extracting procedures adopted might be different among manufacturers, which can increase the variability of crude extract quality. These factors may increase the risk of false-negative diagnosis in the clinical assessment of IgE-mediated food allergy, which could lead to missed diagnosis and delayed treatment.

Over the few past decades, with the increasing application of molecular biology techniques to allergen identification, great advances have been made in food allergen profiling. Currently, it is more common to utilize recombinant DNA technology to produce high quality recombinant allergens in large quantities [4]. These improvements facilitate allergen component-specific IgE testing, which is referred to as component-resolved diagnosis (CRD) or molecular diagnosis of food allergy [5,6]. The CRD approach obviates the drawbacks of conventional crude extract-based methods which had low sensitivity, relatively high false-negativity and cross reactivity [7]. Moreover, CRD allows for high-throughput testing of sIgE to a variety of allergen components simultaneously; this feature facilitates the customization of an individualized allergen component panel, which contributed to individualized desensitization [8]. Currently, only two methods have been approved as CRD methods by FDA: ImmunoCAP (Phadia/Thermo Fisher Scientific, Uppsala, Sweden) and Immulite (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) [9]. Both methods have multiple advantages, including the use of enzyme-catalyzed chemiluminescence, the ability to report results both quantitatively (IU/mL IgE) and semi-quantitatively (7 classes from 0 to 6), a wide detection range, excellent analytical reliability, and satisfactory concordance with skin prick test results. However, the dependence on solid-liquid phase separation and

Abbreviations: LOCI, luminescent oxygen channeling immunoassay; CRD, component-resolved diagnosis; sIgE, specific IgE; ROC, receiver operator characteristic; AUC, the area under the curve; OD, Optical density; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine

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the instability of enzyme activity (e.g., influences from environmental factors such as pH or temperature) are unavoidable drawbacks of these two methods [10,11].

The luminescent oxygen channeling immunoassay (LOCI™) is a homogeneous bead-based method that can resolve the issues mentioned above [12,13]. In a LOCI reaction system, the donor bead can be brought in close proximity (< 200 nm) to the acceptor bead by an antigen-antibody interaction, which enables energy transfer (singlet oxygen) between the two beads and light emission by the acceptor bead [14]. Currently, this technique has been used to detect tumor markers, thyroid hormones, hepatitis B virus antigens and antibodies, interferon-gamma, human insulin, and etc. [15–17]. However, the LOCI technique hasn't been applied to CRD of food allergy so far.

Egg allergy is the second most common food allergy in children. It affects 0.5–2.5% of infants and young children [18]. Children with egg allergy are also at risk of developing other food allergies, including peanut allergy, eczema, and asthma [19]. To date, four major allergens have been well characterized in egg white: ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4) [20]. Of these allergens, ovalbumin shows the highest abundance [21] and is closely associated with allergic diseases in children [22]. Therefore, in the present study, we, for the first time, used ovalbumin as a representative allergen to develop an indirect LOCI system for detecting serum ovalbumin-sIgE. Without use of enzyme or the trouble of separating free and bound tracers, this LOCI system showed excellent performance in measuring ovalbumin-sIgE in terms of precision, sensitivity and anti-interfering ability. Our work provided a novel and excellent method for allergen component-sIgE determination, which contributed to CRD of food allergy.

2. Materials and methods

2.1. Individual samples and ethics statement

A total of 199 individual human serum samples were collected at Tianjin Children's Hospital from January 1, 2015 to December 31, 2015. Of these 199 serum samples, 86 were diagnosed to have positive reaction to egg white (egg white-sIgE values > 0.35 kU_A/L) and the remaining (n = 113) showed negative reaction.

Our study was conducted in accordance with the current version of the Declaration of Helsinki ethical guidelines as well as the national legal and regulatory requirements. The institutional review board of Tianjin Children's Hospital has approved the study protocol, including patient information and consent form. Our study was also approved by the Ethics Committee of Tianjin Medical University. All subjects provided their written informed consent before being enrolled in the study. All samples we collected are leftover serum samples. All traceable identifiers of the samples were removed to ensure anonymous analysis.

2.2. Enzyme-linked immunosorbent assay

ELISA was used for comparative analysis of its performance with that of LOCI. Briefly, ovalbumin dissolved in PBS (10 μg/μL) was added into 96-well plates, with 150 μL each well. Plates were incubated at 37 °C for 3 h, and then at 4 °C overnight to allow ovalbumin to attach completely. Unattached ovalbumin was removed by washing with 0.05% Tween 20/PBS (PBST). After blocking with 5% skimmed milk in PBST at 4 °C overnight, plates were incubated with diluted serum (1:20 dilution) at 37 °C for 1 h, and subsequently, with horseradish peroxidase (HRP)-labeled anti-human IgE antibodies (1:5000 dilution, A9667, Sigma, USA) at 37 °C for another 1 h. After washing the plate with PBST three times, 3,3′,5,5′-tetramethylbenzidine (TMB) was added to react with HRP in the dark for 15 min. Then, 10% H_2SO_4 was added to terminate the reaction. Optical density (OD) values were measured at 450 nm with a microplate reader (BioTek, USA).

2.3. Optimization of conditions

Forty serum samples (20 clinically positive and 20 clinically negative) were randomly selected to determine the OD values with ELISA. According to the results, we pooled samples to form four groups (n = 5 samples/group), as follows: OD values < 0.3 (negative group); OD values < 0.5 (weak positive group); OD values between 1 and 1.5 (positive group); and OD values > 2 (strong positive group). These four groups of pooled sera were utilized to determine optimal conditions for indirect LOCI.

2.4. Luminescent oxygen channeling immunoassay

2.4.1. Indirect LOCI

The indirect LOCI reaction system comprised four components: ovalbumin-conjugated acceptor nanoparticles, biotinylated anti-human IgE antibody, serum, and streptavidin-conjugated donor nanoparticles. The ovalbumin (Sigma, USA) conjugation to acceptor nanoparticles and the biotin (NHS-LC-Biotin; Thermo, USA) labeling of mouse anti-human IgE antibody (Clone GE-1, Sigma, USA) were performed by Beyond Biotech (Shanghai, China). Streptavidin-conjugated donor nanoparticles are a general purpose reagent in LOCI system whose optimal dose (28 μg/mL) had been titrated by Beyond Biotech (Shanghai, China). The special diluent for donor nanoparticles was also provided by Beyond Biotech (Shanghai, China). The diluent for acceptor nanoparticles was 0.1 M Tris-HCl (pH 8.0) and 3% bovine serum albumin (BSA) in H_2O; the serum diluent contained 0.05 M Tris-HCl (pH 7.95–8.05), 0.15 M NaCl, 2% BSA, 0.05% Proclin, and 0.05% Tween20. The procedure included a 2-step reaction. First, serum was incubated with a mixture of biotinylated anti-human IgE antibody and ovalbumin-conjugated acceptor nanoparticles at 37 °C for 45 min. Then, streptavidin-conjugated donor nanoparticles were added for another 15-min incubation in the dark. When sIgE in the serum interacted with ovalbumin, donor and acceptor nanoparticles were brought together (distance less than 200 nm). This allowed energy transfer from donor to acceptor nanoparticles, generating fluorescent light at 610 nm from acceptor nanoparticles (Fig. 1A). Serum sIgE levels were proportionate to fluorescence intensity, which was evaluated with a High Throughput Chemiluminescence Analyzer (LICA HT, Beyond, China).

2.4.2. Sandwich LOCI

We implemented a sandwich mode of LOCI to quantify IgE (Fig. 1B). In the reaction system, the goat anti-human IgE antibody (polyclonal, sigma, USA), instead of ovalbumin in indirect LOCI system, was conjugated to acceptor nanoparticles; IgE was equivalent to an antigen, and a sandwich complex was formed with two anti-human IgE antibodies. A series of sequential dilutions of a human total IgE standard (WHO IgE reference standard 75/502, [23]) were tested separately, to generate a dose curve of concentration vs. fluorescence intensity. The IgE contents in all dilutions were within the expected sIgE detection range.

2.5. Calibration

Following IgE testing by sandwich LOCI, a standard curve was plotted. The calculated concentrations of sequential IgE dilutions were 3, 1.5, 0.75, 0.375, 0.188, 0.094, 0.047, 0.023, and 0 kU_A/L. From the standard curve of concentration (X) vs. fluorescence intensity (Y), a versatile equation was derived based on the ordinary least squares fit method: $Y = A*X\hat{}B + C*X\hat{}D$.

2.6. Parameters for performance evaluation

LOCI performance was evaluated by determining the sensitivity, assay precision, and anti-interference ability. Sensitivity was expressed as the maximum dilution of positive serum that could be distinguished from negative serum with LOCI. Precision was measured as the inter-

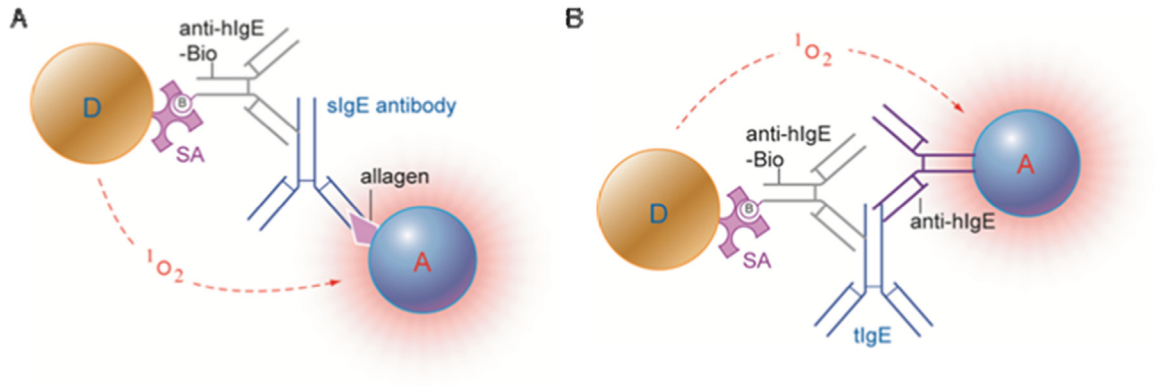

Fig. 1. Schematic diagram of LOCI. (A) Indirect LOCI for determination of sIgE in human serum; (B) Sandwich LOCI for generating a calibration curve for IgE quantification.

assay and intra-assay variability of LOCI, and was expressed as the coefficient of variation (CV = standard deviation ρ/ mean value μ). The CV value was measured by testing a serum sample ten times with a single lot of reagents every day over a period of 10 days. The anti-interference ability was defined as the ability of LOCI to accurately determine ovalbumin-sIgE levels in the presence of interfering factors, such as hemolysis, hyperlipidemia, jaundice, and ascorbic acid.

2.7. Statistics

Data are expressed as the means ± SD of at least two independent experiments performed in duplicate or triplicate. Analyses were performed with GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). The two-tailed student's *t*-test was used to determine the significance of differences between the ELISA and LOCI. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Optimization of the assay

The schematic diagrams of the indirect and sandwich LOCI models are shown in Fig. 1. We used indirect LOCI system to optimize the reaction conditions via the checkerboard titration method. The reaction conditions to be identified included the concentrations of ovalbumin-conjugated acceptor nanoparticles and biotinylated anti-human IgE antibodies, the dilution ratio of serum, and the incubation time (Table 1). We found that the fluorescence intensity ratio of positive serum to negative serum (P/N) reached a peak in all weak positive, positive, and strong positive groups, when the assay was run with a 1/600 dilution (16.67 μg/mL) of ovalbumin-conjugated acceptor nanoparticles (Fig. 2A, Table 1). Additionally, maximal P/N ratios were obtained with a 1/1000 dilution of biotinylated anti-human IgE antibodies (Fig. 2B, Table 1). Hence, 16.67 μg/mL ovalbumin-conjugated acceptor nanoparticles and 1.047 μg/mL biotinylated anti-human IgE antibodies were considered optimal conditions for the LOCI system. Next, we applied these conditions to assess the influence of different serum dilutions on assay performance. We found that the optimum dilution ratio of serum was 1/20 (Fig. 2C). Then, under these three optimal conditions, the incubation time was evaluated. We found that the P/N ratio increased gradually with incubation time and reached a dynamic balance at 45 min (Fig. 2D). Thus, 45 min was deemed the optimum incubation time. These optimum conditions were used in all subsequent work.

3.2. Calibration

Under optimum conditions, we performed sandwich LOCI with serial dilutions of an IgE standard (0, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5, 3 kU_A/L). An IgE standard curve was generated by directly plotting fluorescence signals (Y) against IgE concentrations (X), with no exclusion of calibration points (Fig. 2E). The equation ($Y = A*X\hat{}B + C*X\hat{}D$, A = 3066, B = 0.8506, C = 258.2, D = 4.736e-007; $r^2 = 0.9983$) was obtained from a least-squares curve fitting analysis by GraphPad Prism 5.

3.3. Assay sensitivity

Based on the ELISA results for 40 serum samples (Tables S1–2), we selected four individual allergic serum samples from patients 1, 3, 26, and 44 to evaluate the sensitivity of the indirect LOCI system. These four individuals were selected, because they represented high, intermediate, and low serum levels of ovalbumin-sIgE. One pooled negative sera was used as the control. We found that all allergic sera could be clearly distinguished from the negative sera up to a 40-fold dilution (Fig. 3). However, when using 1/80 dilution of sera, low sIgE levels of patient 3 couldn't be clearly distinguished from negative sera. Thus, the sensitivity of the indirect LOCI was determined to be a 1/40 serum dilution.

3.4. Assay precision

Inter-assay and intra-assay CVs were calculated to evaluate the precision of indirect LOCI system. Four pooled sera with different sIgE levels (negative, weak positive, positive, and strong positive) were analyzed ten times a day over a period of 10 days. The CV value of intra-assay ranged from 0% to 4.43%, and the CV value of inter-assay ranged from 0% to 6.69% (Table 2); both were statistically acceptable (CV ≤ 10%).

3.5. Anti-interference ability

To evaluate the anti-interference ability of indirect LOCI system, we added several potential interfering substances, including bilirubin, triglyceride, ascorbic acid, and hemoglobin, into the reaction system. The recovery rate (RE%) was calculated. We observed that 37.6 mg/dL bilirubin or 600 mg/dL hemoglobin in serum, considered high levels clinically, caused little interference to ovalbumin-sIgE detection with LOCI; under both conditions, the RE% values exceeded 80% (Table 3). However, triglyceride and ascorbic acid exerted some interference to LOCI detection; Intermediate levels of triglyceride (1509 mg/dL) and ascorbic acid (100 mg/dL) in the serum led to RE% values below 80% (Table 3).

Table 1
Checkerboard titration for optimal conditions for LOCI.

Serum sample dilution	Biotinylated anti-human IgE (1047 μg/mL)	Groups	Acceptor-Albumin(10 mg/mL)		
			1:200 (50 μg/mL)	1:600 (16.67 μg/mL)	1:900 (11.11 μg/mL)
1:10	1:500	Negative	502.5 ± 12.02	152.5 ± 24.75	73 ± 7.07
		Weak positive	1100 ± 93.34	404 ± 0	156.5 ± 6.36
		Positive	2875.5 ± 181.73	1216 ± 7.78	523.5 ± 34.65
		Strong positive	11644 ± 779.23	4067.5 ± 14.50	1235.5 ± 77.07
	1:1000	Negative	481 ± 0	153 ± 2.83	56.5 ± 0.71
		Weak positive	933 ± 24.04	343.5 ± 4.95	116 ± 41.01
		Positive	2863.5 ± 27.58	1281 ± 94.75	407 ± 107.48
		Strong positive	11943 ± 11.31	3886.5 ± 81.32	907.5 ± 12.02
	1:2000	Negative	463.5 ± 40.31	173 ± 4.24	151.5 ± 109.60
		Weak positive	802 ± 21.21	252 ± 46.67	115 ± 1.41
		Positive	3076 ± 114.55	1233.5 ± 164.76	566 ± 14.14
		Strong positive	11296.5 ± 836.51	3415 ± 675.99	1255 ± 22.63
1:20	1:500	Negative	497.5 ± 16.26	173 ± 18.38	135.5 ± 13.44
		Weak positive	915 ± 0	327 ± 3.54	122 ± 7.07
		Positive	2303.5 ± 78.49	1175.5 ± 11.67	461 ± 39.60
		Strong positive	10290.5 ± 328.80	3976 ± 49.50	1325.5 ± 84.15
	1:1000	Negative	592.5 ± 102.53	142 ± 28.28	80 ± 14.14
		Weak positive	842 ± 39.60	284 ± 25.46	107 ± 0
		Positive	2261.5 ± 27.58	1160.5 ± 74.25	411 ± 97.58
		Strong positive	10327 ± 90.51	3589 ± 224.86	735.5 ± 176.07
	1:2000	Negative	503 ± 0	152.5 ± 14.85	71.5 ± 6.36
		Weak positive	683 ± 12.73	239.5 ± 6.36	110.5 ± 19.09
		Positive	2352 ± 26.87	1159.5 ± 6.36	472.5 ± 55.86
		Strong positive	9416.5 ± 403.76	3498.5 ± 194.45	1118.5 ± 64.35
1:40	1:500	Negative	521 ± 87.68	169.5 ± 0.71	82 ± 12.73
		Weak positive	712.5 ± 10.61	255.5 ± 6.72	108 ± 2.83
		Positive	1541.5 ± 26.16	830.5 ± 15.20	356.5 ± 19.09
		Strong positive	7284.5 ± 157.68	3066 ± 6.36	1077.5 ± 27.58
	1:1000	Negative	572.5 ± 41.72	162.5 ± 2.12	55.5 ± 3.54
		Weak positive	663 ± 79.20	238.5 ± 19.09	86 ± 29.70
		Positive	1618.5 ± 4.95	895.5 ± 7.78	214 ± 19.80
		Strong positive	6923 ± 108.89	2914.5 ± 120.92	663 ± 18.38
	1:2000	Negative	464.5 ± 41.72	167 ± 2.83	75 ± 1.41
		Weak positive	622 ± 36.77	208 ± 14.14	83.5 ± 13.44
		Positive	1552 ± 65.05	803.5 ± 44.55	324.5 ± 9.19
		Strong positive	6635 ± 83.44	2860.5 ± 101.12	710 ± 270.11

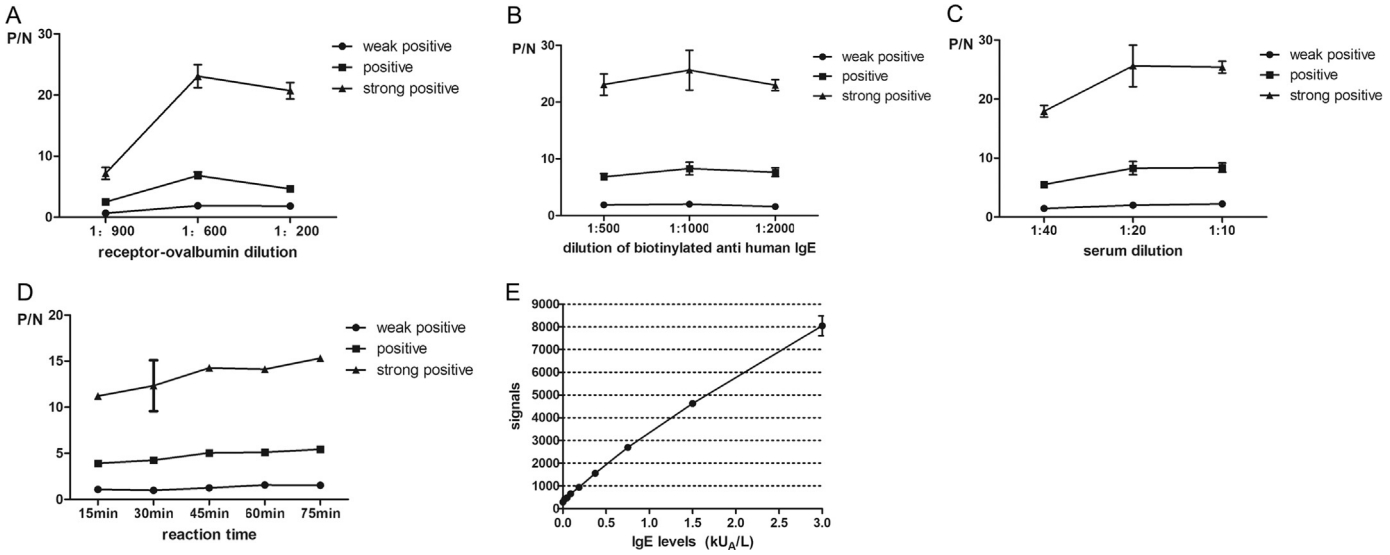

Fig. 2. The optimum conditions. (A, B, C) Evaluation of the optimal dilution ratios of ovalbumin-conjugated acceptor nanoparticles (A), biotinylated anti-human IgE antibodies (B) and serum (C). Two independent experiments were conducted and shown is a representative result with four replicates. P/N, the fluorescence intensity ratio of positive serum to negative serum; weak positive (< 0.5), positive (1–1.5) and strong positive (> 2.0), divided by ELISA. The concentrations of ovalbumin (A) and biotinylated anti human IgE antibodies (B) in stock solution are 10 and 1.047 mg/mL, respectively. (D) Evaluation of the optimal incubation time. Two independent experiments were conducted and shown is a representative result with four replicates. P/N, the fluorescence intensity ratio of positive serum to negative serum; weak positive (< 0.5), positive (1–1.5) and strong positive (> 2.0), divided by ELISA. (E) The calibration curve for determination of ovalbumin-sIgE. Different concentrations of IgE (0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5, 3 kU_A/L) serially diluted from an IgE standard (3 kU_A/L) were used to generate a standard curve. $r^2 = 0.9983$.

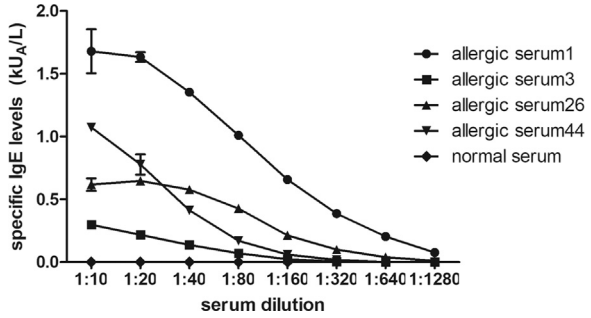

Fig. 3. The assay sensitivity. Individual serum samples from Patient 1, 3, 26, 44 and one pooled negative sera were selected for evaluation of assay sensitivity.

Table 2
Assay precision: intra-assay and inter-assay of the indirect LOCI.

Group	Intra-assay precision (n = 10)		Inter-assay precision (n = 10)	
	Measured(kU$_A$/L)[a]	CV (%)[b]	Measured(kU$_A$/L)[a]	CV (%)[b]
Negative	0 ± 0	0	0 ± 0	0
Weak positive	0.03 ± 0.001	3.33	0.03 ± 0.002	6.69
Positive	0.20 ± 0.01	4.43	0.19 ± 0.009	4.74
Strong positive	0.95 ± 0.04	4.43	0.94 ± 0.044	4.68

Abbreviation: CV, coefficient of variation.
[a] Mean value ± standard deviation (SD).
[b] CV = (SD/Mean) × 100%.

3.6. Comparison of LOCI with ELISA

We performed ROC analyses to compare the performances of indirect LOCI and ELISA. The areas under the curves (AUCs) for indirect LOCI and ELISA were 0.907 and 0.657, respectively (Fig. 4). These values indicated that the analytical performance of the LOCI was superior to that of the ELISA. Additionally, because the LOCI method could generate fluorescence signals that spanned 5 orders of magnitude, it was much easier to distinguish between positive and negative sera compared to the ELISA method (Fig. 5).

4. Discussion

LOCI is a chemiluminescence-based homogenous immunoassay that was first established by Breslow et al. in 1994 [24]. It takes advantage of antigen-antibody interactions to bring donor and acceptor nanoparticles together (< 200 nm), which facilitates singlet oxygen diffusion between the two nanoparticles, causing fluorescence emission by acceptor nanoparticles [25]. The LOCI method saves the trouble of separating free and bound nanoparticles, which makes it superior to heterogeneous assays, such as ELISA, radioimmunoassay, and immunoblotting [26]. Moreover, the simplified procedure of LOCI makes it easy to be automated [27]. In our study, the indirect LOCI system comprised ovalbumin-conjugated acceptor nanoparticles, biotinylated anti-human IgE antibody, ovalbumin-sIgE, and streptavidin-conjugated donor nanoparticles. We demonstrated that this system exhibited excellent performance, and its analytical performance was superior to that of the ELISA.

The present study had some limitations. First, the cost for conjugation of ovalbumin to acceptor nanoparticles was relatively high, due to the lack of generality. Thus, we prioritized the optimization of ovalbumin-conjugated acceptor nanoparticles. Additionally, there was a possibility that other IgE in the serum might compete with ovalbumin-sIgE for binding to biotinylated anti-human IgE antibodies. This could lead to the loss of a large amount of anti-human IgE antibodies, and the light signal generated would be reduced. However, that scenario was unlikely to happen in the present study, because 1/1000 dilution (1.047 μg/mL) of anti-IgE antibodies provided an excess for binding to total IgE in 20-fold diluted serum [28]. Similarly, 20 μg/mL of streptavidin-conjugated donor nanoparticles was also sufficient to bind all biotins in the ovalbumin-sIgE-anti-IgE antibody complex. Thirdly, due to the relatively large molecular weight of antibodies (about 150 KD), there was a possibility that the antibody-IgE-antibody complex in sandwich LOCI model might be too large (> 200 nm) to allow for an energy transfer. However, this possibility was excluded because light signals at 610 nm can be easily captured by the Chemiluminescence Analyzer. We inferred that the three antibodies might form a stereoscopic structure that showed a spatial distance within 200 nm, just like the structure in Fig. 1B. This structure makes sandwich LOCI model workable.

Table 3
Interference from supplement of bilirubin, triglyceride, ascorbic acid and hemoglobin in allergic serum samples.

Interfering Substance	Concentrations in serum	Signals		Recovery (%)	
		Serum sample 43	Serum sample 21	Serum sample 43	Serum sample 21
Bilirubin(mg/dl)	0	855 ± 72.12	1611 ± 39.6	100	100
	4.8	748 ± 27.30	1556 ± 44.20	87.49	96.59
	9.4	747 ± 24.53	1443 ± 1.74	87.37	89.57
	37.6	644 ± 8.49	1352 ± 33.23	75.32	83.92
Triglyceride(mg/dl)	0	855 ± 72.12	1611 ± 39.6	100	100.00
	754.4	692 ± 18.40	1421 ± 48.28	80.94	88.21
	1509	629 ± 24.58	1377 ± 80.88	73.57	85.47
	3017.8	620 ± 38.18	1330 ± 145.66	72.51	82.56
Ascorbicacid(mg/dl)	0	855 ± 72.12	1611 ± 39.6	100	100.00
	50	708 ± 35.24	1412 ± 130.47	82.81	87.65
	100	669 ± 31.77	1312 ± 52.35	78.25	81.44
	200	655 ± 75.29	1035 ± 10.82	76.61	64.25
Hemoglobin (mg/dl)	0	855 ± 72.12	1611 ± 39.6	100	100.00
	75	782 ± 126.53	1579 ± 107.75	91.46	98.01
	150	727 ± 74.12	1447 ± 105.86	85.03	89.82
	600	708 ± 4.95	1446 ± 4.24	82.81	89.76

[a]The concentration used for indirect LOCI after dilution.
[b]The corresponding concentration of clinical serum sample before dilution.
[c]The commonly used unit in clinic.
The initial concentration of bilirubin, triglyceride, ascorbic acid and hemoglobin is 64.4umol/L, 17.05 mmol/L, 200 mg/mL and 3 g/L, respectively. The serum samples for interference test is selected randomly in egg white allergic children. The average signals of sample 43 and 21 with no addition of interfering substance is 855 and 1611, respectively.

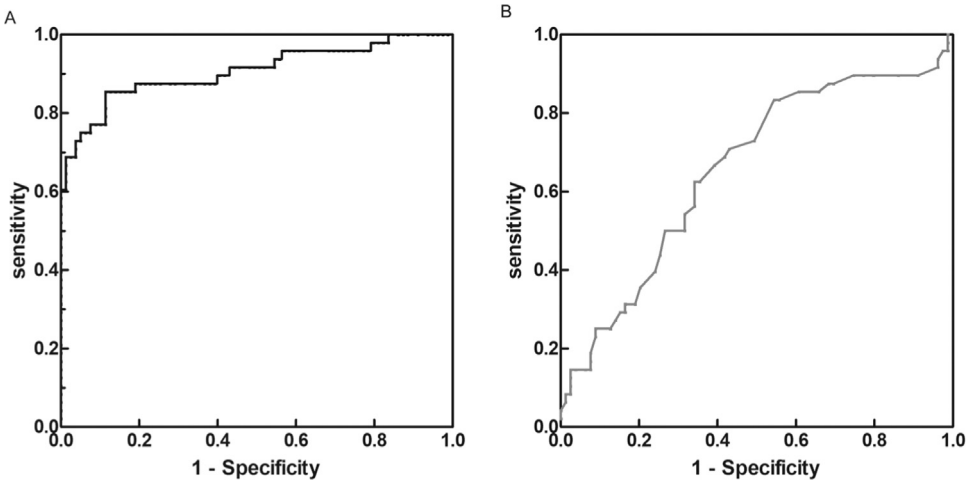

Fig. 4. ROC analysis. (A, B) Comparison of Indirect LOCI (A) with ELISA (B) for determination of serum ovalbumin-sIgE by plotting ROC curves. ROC, receiver operating characteristic curve. The area under the curve (AUC) is 0.907 for LOCI (A) and 0.657 for ELISA (B).

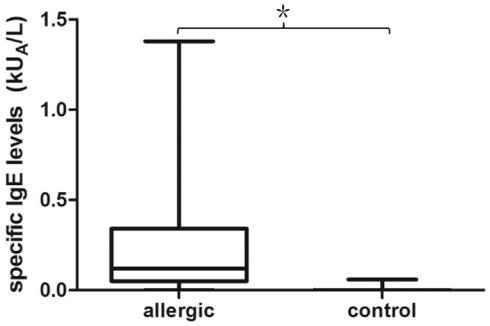

Fig. 5. Box-and-whisker plots of serum ovalbumin-sIgE levels in allergic and control groups measured by using LOCI method. The box indicates the 25th, 50th and 75th percentile, and the error bars indicate the upper and lower extremes of measured concentrations. *, $P < 0.05$.

In the present study, the indirect LOCI system showed high sensitivity. A 40-fold dilution of serum had little influence on the detection efficiency of the assay. The Hook effect, which often occurs in ELISA tests, could be avoided with the LOCI method, because the LOCI reaction system allowed a high load of ovalbumin, due to the large areas and the suspension properties of nanoparticles [29,30]. Some egg-allergic sera exhibited low fluorescence signals. This finding could be explained by the fact that these patients were primarily allergic to egg components other than ovalbumin. The fluorescence intensities with LOCI covered a larger range of magnitudes than the optical density range covered with ELISA; thus, positive and negative sera were easier to be differentiated with the LOCI method [31].

In the present study, the indirect LOCI system showed good precision, because it did not require any washing steps in the operational process or have environment-sensitive enzymes in the reaction system. Our ROC analysis results demonstrated the superiority of LOCI over ELISA. However, because the LOCI was a homogenous system, this assay is potentially vulnerable to plasma interference from several sources, including inner filter effects, autofluorescence, bilirubin, hemolysis, triglycerides, and ascorbic acid [32]. The inner filter effects can be minimized by using Eu^{3+} (with a emission wavelength of 614 nm) as the fluorophore loaded into the acceptor nanoparticles [24]. Additionally, Eu^{3+} has a long fluorescence lifetime and a large Stokes shift. Therefore, Eu^{3+} measurements can be carried out in a time-resolved manner to eliminate interference from autofluorescence [33]. Hemolysis and bilirubin were shown to cause little interference in assay analysis. However, the assay results were influenced by medium and high blood levels of triglyceride and ascorbic acid. Because triglyceride promotes light scattering and ascorbic acid is a reducing agent [34], we inferred that the weakened fluorescence signal was caused by triglyceride-induced scattering of light and ascorbic acid-induced quenching of the singlet oxygen.

5. Conclusions

In this study, we established a LOCI system method for determining ovalbumin-sIgE in human serum. This assay exhibited excellent performance, superior to the performance of ELISA, in terms of analytical quality, cost effectiveness, and operation difficulty. In future, different component-conjugated acceptors can be combined in this reaction system and measured simultaneously, thus allowing multiple allergen component-sIgE determination. All in all, our findings will contribute to CRD of food allergy.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jlumin.2018.04.011.

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