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RESEARCH ARTICLE

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Performance evaluation of a laboratory-developed light-initiated chemiluminescence assay for quantification of egg white-specific IgE

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Abstract

Background: Specific IgE (sIgE) testing has become one of the most important tools for diagnosing IgE-mediated food allergy. Enzyme-linked immunosorbent assay (ELISA) and dot-enzyme-linked immunosorbent assay (Dot-ELISA) have been used to measure sIgE in clinical widely. Light-initiated chemiluminescence assay (LICA) is a new method for measuring allergen-sIgE. We aimed to establish a LICA method for quantitative detection of egg white-sIgE and evaluate its performances.

Methods: The best chemibeads coupling method in detecting egg white-sIgE was selected, and a LICA method for quantitative detection of egg white-sIgE was established. The precision study was performed according to Clinical and Laboratory Standards Institute (CLSI) EP5-A2. Detection capability which contains limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) was evaluated according to National Health Commission of the People's Republic of China (NHC) WS/T 514–2017. Linear range was evaluated according to CLSI EP6-A. All data were analyzed using SPSS software.

Results: Precision contains repeatability and intermediate precision. The CV of repeatability ranged from 2.72% to 7.29%, and the CV of intermediate precision ranged from 4.93% to 8.64%. The LoB, LoD, and LoQ of the assay were 0.000 kUA/L, 0.053 kUA/L, and 0.076 kUA/L. The assay linear range was 0.076–34.125 kU_A/L ($r = 0.9979 \geq 0.9900$).

Conclusion: This laboratory-developed LICA method can detect egg white-sIgE, and performance meets clinical requirements. This method shows rapid turnaround cycles and high sensitivity. It can be used as an alternative method for clinical detection of egg white-sIgE.

KEYWORDS
egg white-specific IgE, evaluation, light-initiated chemiluminescent assay, performance, quantification

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1 | INTRODUCTION

Egg allergy is a pervasive condition affecting up to 9% of children worldwide.[1,2] The burden of the disease is severe during early childhood as symptoms include vomiting, abdominal pain, diarrhea, and urticaria.[3,4] Egg-allergic patients strictly avoid all egg products and cause malnutrition because eggs can provide essential vitamins, proteins, and fatty acids.[5] Previous article has suggested that the majority of egg-allergic infants and children are sensitized to egg white but not egg yolk allergens.[6] Distinguishing between egg white and egg yolk allergy can help patients accurately avoid the allergic food. So, early diagnosis and therapy with egg white allergy appear to be particularly important. The diagnostic approach of egg allergy is based on the clinical history and the presence of specific IgE (sIgE).[7] The sIgE testing has become one of the most important tools for diagnosing IgE-mediated food allergy.[8,9]

Currently, there are several methods for detecting allergen-sIgE in vitro. The "gold standard" for in vitro diagnosis of allergic conditions is ImmunoCAP system,[10] which is a fluorescence enzyme immunoassay (FEIA). Enzyme-linked immunosorbent assay (ELISA) and dot-enzyme-linked immunosorbent assay (Dot-ELISA) have been used in clinical widely.[11,12]

Dot-ELISA is a semi-quantitative method for measuring allergen-sIgE. The nitrocellulose membrane (NC membrane) is coated with multiple allergens at multiple locations, which can detect multiple sIgE at the same time and meet the requirements of early clinical allergen screening.[13] The detection cost is inexpensive. But, this method also has some disadvantages, such as long test duration, non-quantitative, and low sensitivity. ELISA is a quantitative method for allergen-sIgE measurement. The ELISA plate is coated with anti-human IgE antibody, and it is convenient that biotinylated antigen can be changed according to different allergen tests. The use of avidin and biotin systems can amplify signal and then improve the analytical sensitivity. Compared with Dot-ELISA, ELISA is more suitable for quantitative analysis.[14] The disadvantage is that due to the limitation of ELISA plate coating area, high amount of total IgE (tIgE) may affect the binding of sIgE antibody so that influence the sensitivity of analysis. ImmunoCAP system has strong anti-interference capability to allergen-specific IgG antibodies. Furthermore, ImmunoCAP system has high analytical sensitivity.[15] But, it has long turnaround time and is not widely used in China because of expensive detection price.

Light-initiated chemiluminescence assay (LICA) is a homogeneous chemiluminescence immunoassay based on singlet oxygen transmission. The application of nanospheres can couple with large amounts of antigen so that has good binding capacity and avoids interference of allergen-specific IgG.[16,17] It exhibits rapid turnaround cycles, high sensitivity, and excellent reproducibility.[18] Therefore, this study will establish a LICA method for the detection of egg white-sIgE and pay attention to the performance evaluation according to Clinical and Laboratory Standards Institute (CLSI) EP5-A2,[19] CLSI EP6-A,[20] CLSI EP17-A,[21] and National Health Commission of the People's Republic of China (NHC) WS/T 514–2017.[22]

2 | MATERIALS AND METHODS

2.1 | Samples

A total of 103 serum samples were collected from Tianjin Children's Hospital from January 2021 to March 2022. Sixty-eight samples which sIgE were higher than 0.35 kU_A/L by ELISA (HOB, China) were diagnosed as egg allergy. Control samples ($n = 35$) were nonallergic individuals (sIgE < 0.35 kU_A/L). Informed consent was obtained from all patients. No personal information was obtained in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Our study was approved by the Ethics Committee of the Tianjin Children's Hospital (TMUHMEC2017008).

2.2 | Reagents

Egg white allergen Gal d1-d4 was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Biotinylated goat anti-Human IgE antibody was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Chemibeads and streptavidin-coated sensibeads were obtained from Beyond Biotech (Shanghai, China).

2.3 | Establishment of a LICA method for quantitative detection of egg white-sIgE

2.3.1 | Coupling of egg allergens to chemibeads

There were two coupling methods of chemibeads. One was first prepared a mixture of four egg white components (Gal d1, Gal d2, Gal d3, and Gal d4) and then coupled the combined mixture on chemibeads (called method 1 in the following article). And, the other was first coupled with four kinds of chemibeads and combined them as mixture chemibeads (method 2). The mixture of four egg white components or a single egg white component was coupled to chemibeads using similar steps as previously described.[18] In brief, the chemibeads were washed twice with ultrapure water before use. Phosphate buffer solution (PBS)-dissolved components were added to carbonate buffer solution (CBS) suspended chemibeads. The complex was rotated at 37°C overnight. 8 mg/ml NaBH4 and 75 mg/ml glycine were successively added to the complex, and the reaction mixture was spun at room temperature. Finally, the conjugate was centrifuged, washed, and suspended in a storage buffer (containing 25 mM HEPES, 1% BSA and 0.1% Proclin). The final concentration of chemibeads was 10 mg/ml, and chemibeads were stored in 4°C.

To determine the best coupling method in detecting egg white-sIgE, nine serum samples which clinical values ranged from 0.11 to 0.34 kU_A/L were collected and simultaneously detected with chemibeads of method 1 and method 2 by LICA. The serum from nonallergic individual was used as the negative control. The sample serum and negative serum were designated as S and N, respectively.

The chemiluminescence intensity ratio of sample serum to negative serum (S/N ratio) was calculated.

2.3.2 | Optimization of the reaction conditions of LICA for the detection of egg white-sIgE

Method 1 was confirmed as the best coupling method of chemibeads, so we only optimized mass ratio and dilution ratio of chemibeads which were labeled with method 1. First, three mass ratios of chemibeads of method 1 (80:1, 20:1, 10:1) were prepared. Then, nine serum samples which sIgE clinical values ranged from 0.11 to 0.34 kU_A/L were collected. Sera from nonallergic individuals served as negative controls. The egg white-sIgE of each sample was detected by LICA. S/N ratio of each serum was calculated to obtain the optimal mass ratio. To optimize the best dilution ratio, two serum pools containing a high and low level of sIgE were selected. Sera from nonallergic individuals served as negative controls. The chemibeads with best mass ratio were diluted at 1:200, 1:400, and 1:800. The two serum pools were detected by LICA. S/N ratio of each serum was calculated, and the best dilution ratio of chemibeads was determined.

To get the best buffer system of chemibeads labeled with method 1, seven buffer systems included phosphate buffer (PB), phosphate buffer saline (PBS), carbonate buffer solution (CBS), HEPES buffer, Tris–HCl buffer, sodium citrate buffer, and MES buffer were selected. Each buffer system was prepared to the most common concentration and pH value. Four serum pools containing a high, middle, low, and very low level of sIgE were selected and detected by LICA. Sera from nonallergic individuals served as negative controls. The S/N ratio of each serum was calculated. Then, pH value and concentration of PB were optimized because PB was chosen as the buffer system of chemibeads. Three pH values containing 6.8, 7.4, and 8.0 of PB were prepared. Serum pools were selected the same as for buffer system selection. The S/N ratio of each serum was calculated to obtain the best pH value. Three concentrations containing 0.01 M, 0.025 M, and 0.05 M PB with the best pH value were prepared. Serum pools were selected the same as for buffer system selection. The S/N ratio of each serum was calculated to obtain the best concentration.

Three surfactants were selected to add to the buffer system to explore whether they are useful for improving the S/N ratio. PB with 0.01% Triton X-100, 0.01% Tween 20, and 0.01% sodium deoxycholate were prepared. Serum pools were selected the same as for buffer system selection. The S/N ratio of each serum was calculated to obtain the best surfactants.

To obtain the best dilution ratio of biotinylated goat anti-human IgE antibody, the antibody was serially diluted at 1:1000, 1:2000, and 1:4000. The serum which sIgE concentration was 28 kUA/L was selected and diluted to four different concentrations. Sera from nonallergic individuals served as negative controls. S/N ratio of each serum was calculated.

To obtain a calibration curve, the serum which concentration was 45.5 kU_A/L (measured by ImmunoCAP system) was selected and sequentially diluted at 17.5, 3.5, 0.7, and 0.35 kU_A/L. Serum from nonallergic individual was used as 0 kU_A/L. All 6 calibrators were detected by LICA. The concentration and corresponding chemiluminescence signal (CL signal) were used to plot a calibration curve based on four-parameter logistic regression. The concentration of the calibrator was further confirmed by parallel comparison of 15 serums with ImmunoCAP system.

2.4 | Performance evaluation of LICA for the detection of egg white-sIgE

2.4.1 | Precision

Precision was evaluated by repeatability and intermediate precision. Three serum pools containing a high, middle, and low level of egg white-sIgE were selected. According to CLSI EP5-A2,[19] for repeatability, each sample was measured 20 times in an analytical run. For intermediate precision, each sample was measured 8 times per day for five consecutive days.

2.4.2 | Limit of blank, limit of detection, and limit of quantitation

According to NHC WS/T 514–2017,[22] to detect LoB, five nonallergic individual serum pools were collected. Each sample was measured four times per day for three consecutive days. To detect LoD, four serum pools in which egg white-sIgE ranged from 1 to 4 LoB were collected. Each sample was measured five times per day for three consecutive days. To detect LoQ, the serum which sIgE was 0.604 kU_A/L was selected and diluted to 0.302, 0.151, and 0.076 kU_A/L. Each concentration was measured ten times in one run.

2.4.3 | Linearity

According to CLSI EP6-A,[20] two serum pools containing a high and low level of egg white-sIgE were selected and prepared to seven concentrations (0.076–34.125 kU_A/L). Each concentration was measured three times in one run, and the standard deviation (SD) was calculated.

2.5 | Method comparison

All 68 egg-allergic patients and 35 nonallergic individuals participated in this study. Commercial ELISA kit (HOB, China) was used for comparison with LICA, and all sera were measured for egg white-sIgE

with two methods. Positive coincidence rate, negative coincidence rate, and total coincidence rate were calculated.

2.6 | Statistical analysis

Differences between groups were analyzed using paired Wilcoxon signed-rank test and Friedman test with SPSS (version 25, SPSS Inc., Chicago, USA). $p < 0.05$ indicated a statistically significant difference.

3 | RESULTS

3.1 | Mixture of egg white components coated on sensibeads is better than combined the single-coated beads methods

Chemibeads of method 1 showed higher median S/N ratios in comparison with method 2, and the result is shown in Figure 1A,B. Method 1 chemibeads displayed better performance in detecting low-value egg white-sIgE serum.

3.2 | Optimization of the reaction conditions

When method 1 mass ratio was 20:1, the mean of S/N ratio showed highest, so we chose 20:1 as the optimal mass ratio of chemibeads (Figure 2A). We diluted the chemibeads at ratios of 1:200, 1:400, and 1:800. The results showed that when the dilution was 1:200, the S/N ratios of two sera were not significantly improved but the background signal was increasing (not shown). Therefore, 1:400 was chosen as the optimal dilution of the chemibeads (Figure 2B). The dilution ratios of biotinylated goat anti-human IgE were 1:1000, 1:2000, and 1:4000. The results showed that the S/N ratio showed a little lower in 1:2000 than 1:1000 dilution ratio. In view of economic efficiency, 1:2000 dilution was chosen as the optimal condition of the biotinylated goat anti-human IgE (Figure 2C).

As shown in Figure 3A,B, PB showed the highest S/N ratio and CL signal among several buffer systems, and the background signal was lower than others. Therefore, PB was chosen as the optimal buffer system. When the pH value of PB was 7.4, the S/N ratios of four serum pools were highest and the background signal was lower than others. Therefore, the optimal pH value of PB was 7.4 (Figure 3C,D). We prepared 0.01 M, 0.025 M, and 0.05 M PB. The results showed that the S/N ratios of 0.01 M PB and 0.025 M PB were similar, but the CL signals were higher in 0.01 M PB. Finally, 0.01 M PB was chosen as the optimal concentration (Figure 3E,F). The addition of surfactant improved the S/N ratios of four serum pools, especially in high and middle levels of sIgE serum pools. The results showed that the test got the highest S/N ratio with 0.01% Tween 20 (Figure 3G,H), which was chosen as the optimal surfactant.

As shown in Figure 4, the equation of calibration curve of egg white-sIgE was

$$y = \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}} + D$$

(A = 111869.3, B = −1.1, C = 23.3, D = 977.0, R^2 = 1.0000), which corresponded concentration range is 0.076–34.125 kU_A/L.

3.3 | Performance evaluation of LICA for the detection of egg white-sIgE

3.3.1 | Precision

Average measured value (kU_A/L), SD, and coefficients of variation (CV) were calculated for repeatability and intermediate precision. Separate calculation was performed for each concentration, and all data checked against the outlier criterion which is defined as exceeding 4 times the standard deviation (SD).[23] As shown in Table 1, for the repeatability, the CV values ranged from 2.72% to 7.29%. For the intermediate precision, the CV values ranged from

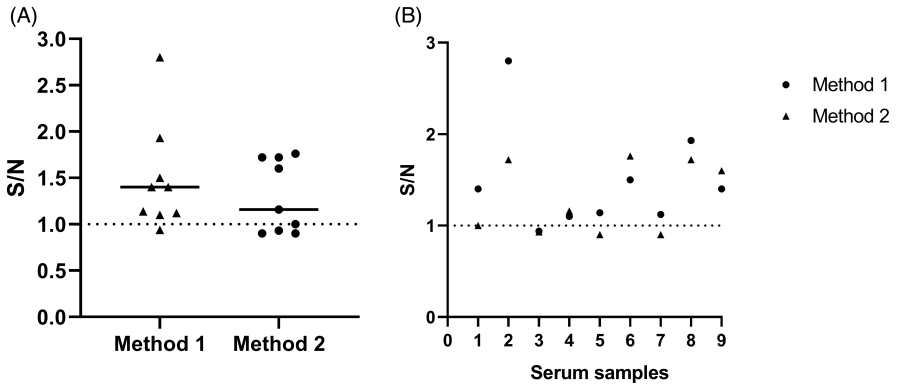

FIGURE 1 Comparison of coupling method 1 and method 2. Nine serum pools which clinical values ranged from 0.11 to 0.34 kU_A/L were measured with chemibeads of two coupling methods, respectively, by indirect light-initiated chemiluminescence assay (LICA). (A) A summary of two methods S/N ratios. Two methods showed no statistical difference analyzed by paired Wilcoxon signed-rank test. (B) Each serum pools S/N ratios of two methods

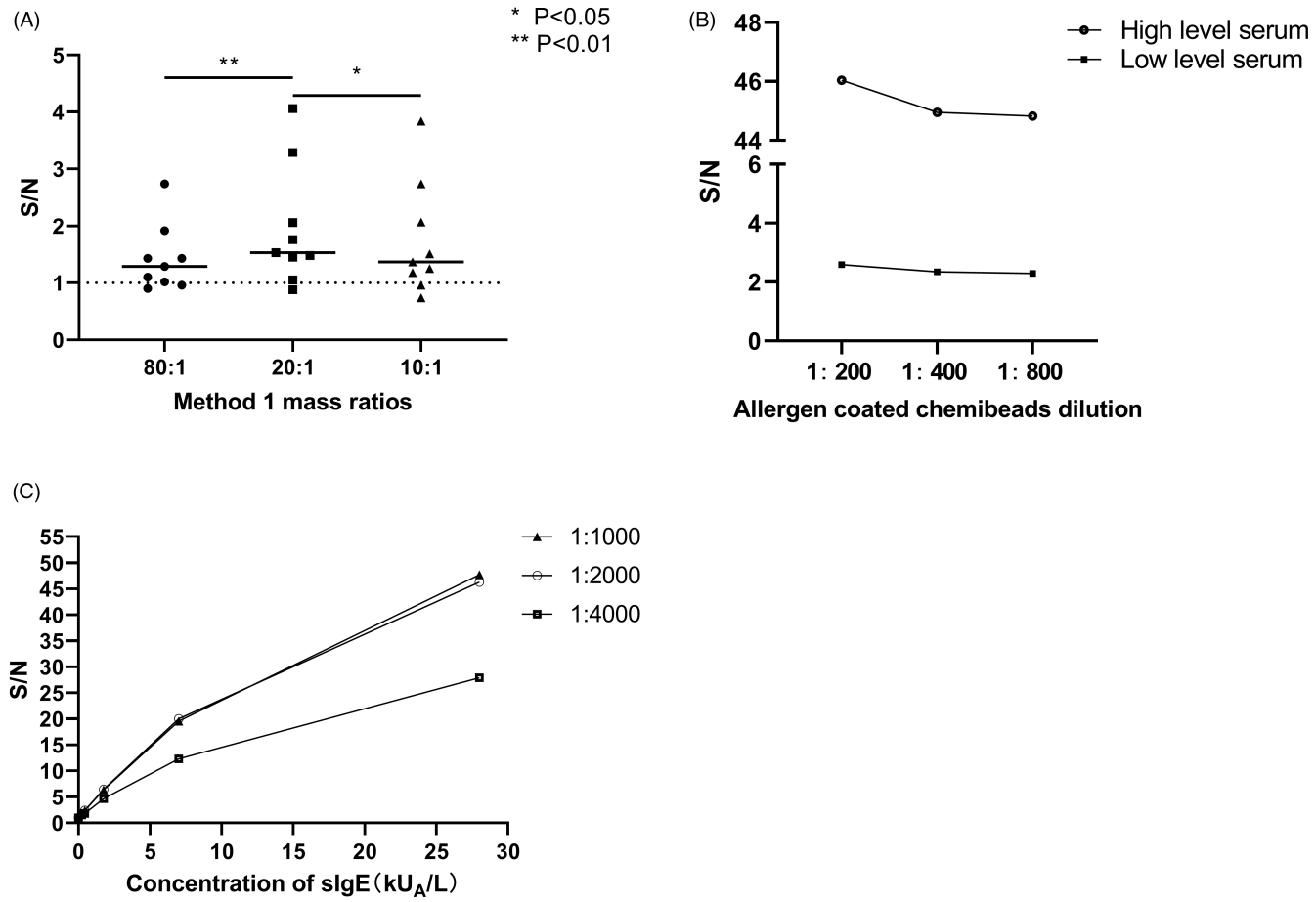

FIGURE 2 Reaction conditions optimization. (A) A comparison of three mass ratios chemibeads which ranged from 80:1 to 10:1. Differences between groups were analyzed using Friedman test. $p<0.05$(*) and $p<0.01$(**) indicated a statistically difference. (B) The effect of different dilution ratios of chemibeads. (C) The effect of different dilution ratios of anti-Human IgE antibody

4.93% to 8.64%. A CV value of less than 10% (intra-assay CV) and 15% (inter-assay CV) was regarded as acceptable.

3.3.2 | Limit of blank, limit of detection, and limit of quantitation

The LoB data showed Gaussian distribution and were calculated with the following formula $LoB = M_B + \frac{1.645}{1-\frac{1}{4(B-K)}} \times SD_B$.[22] M_B and SD_B are the mean and standard deviation of the blank measurements. B and K are the members of the whole blank values and blank sera, respectively. The LoB of the assay was 0.000 kUA/L.

Nonparametric statistical methods were applied on account of the LoD data showing non-Gaussian distribution. LoD was defined as the median of the whole values on the condition that the percentage of observations below the LoB was less than β-percentiles.[22] The LoD of the assay was 0.053 kUA/L.

Limit of quantitation was defined as the minimum concentration, which CV < 15% and cannot be lower than LoD.[21] The LoQ of the assay was 0.076 kUA/L.

3.3.3 | Linearity

The linear range was defined by the highest and lowest measured concentrations where the response was linear.[20] Data were expressed as the means ± SD. The assay linear range was 0.076–34.125 kU_A/L ($r = 0.9979 \geq 0.9900$) (Figure 5).

3.4 | Method comparison

The established LICA was compared with a commercial ELISA (HOB, China). As shown in Table 2, the positive coincidence rate of LICA was 94.12% and the negative coincidence rate was 88.57%, and the total coincidence rate was 92.23%.

4 | DISCUSSION

Light-initiated chemiluminescence assay is a homogeneous immunoassay based on singlet oxygen transmission, which requires no

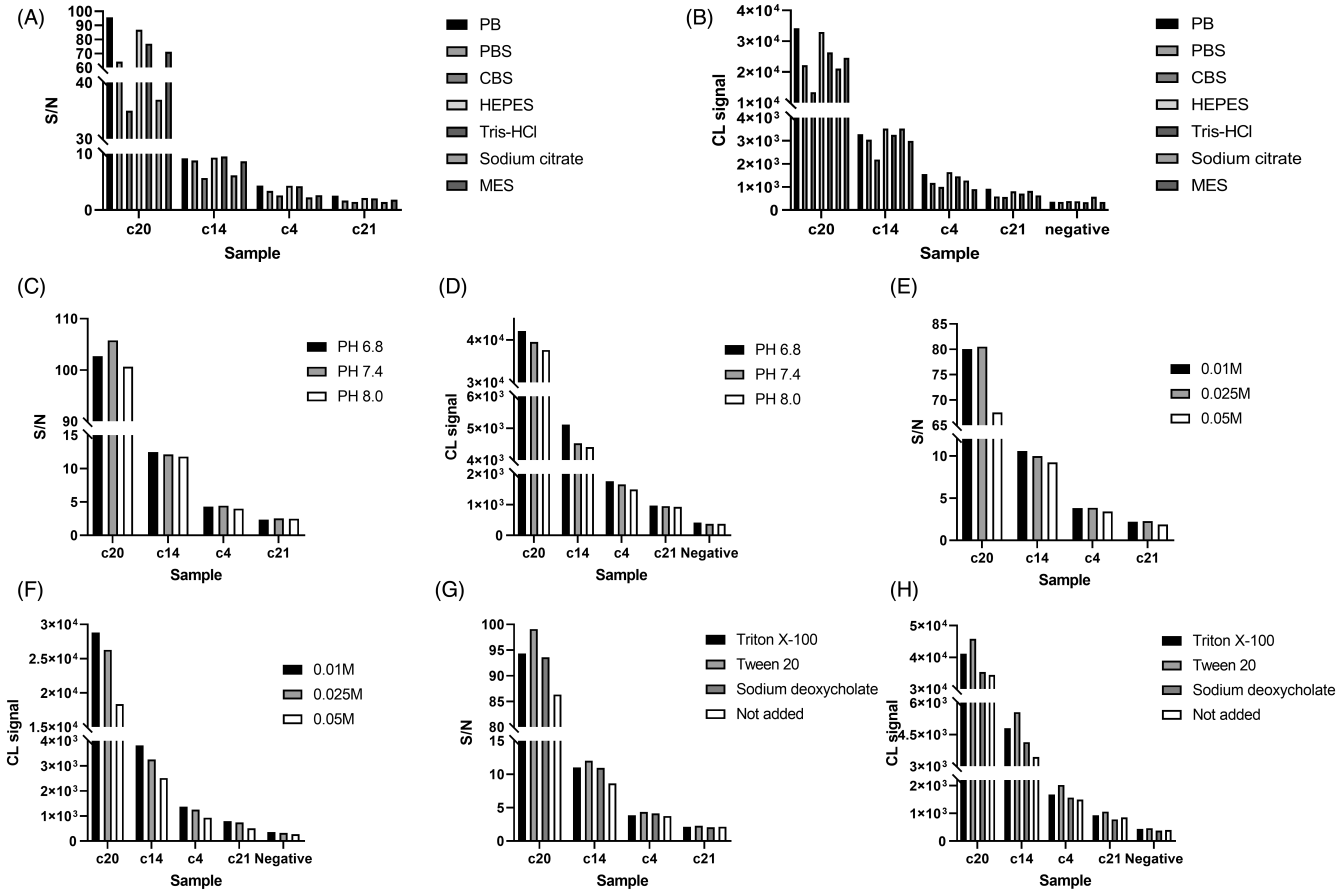

FIGURE 3 Buffer system optimization. (A) The S/N ratios of four serum pools in seven buffer systems. (B) The chemiluminescence (CL) signals of four serum pools in seven buffer systems. (C) The S/N ratios of four serum pools in phosphate buffer (PB) with three pH values. (D) The CL signals of four serum pools in PB with three pH values. (E) The S/N ratios of four serum pools in PB (pH 7.4) with three concentrations. (F) The CL signals of four serum pools in PB (pH 7.4) with three concentrations. (G) The S/N ratios of four serum pools in 0.01 M PB (pH 7.4) with three surfactants. (H) The CL signals of four serum pools in 0.01 M PB (pH 7.4) with three surfactants

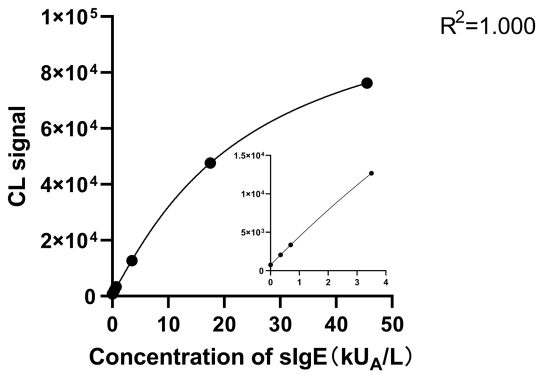

FIGURE 4 Calibration curve of light-initiated chemiluminescence assay (LICA) for detecting egg white-sIgE in human sera based on four-parameter logistic equation. The corresponded concentration range is 0–45.5 kU_A/L.

washing steps. It can overcome shortcomings such as enzyme instability in ELISA[24] and the long turnaround time existing in FEIA. Moreover, LICA eliminates the need for complex processes to prepare reagents. Allergen-coupled chemibeads have simple standardized steps; biotinylated anti-human IgE antibody and streptavidin-coated sensibeads are commercial products. Indirect LICA was established in this study for the detection of egg white-sIgE. There are two egg white allergen coupling methods of chemibeads. One is first preparing a mixture of four egg white components and then coating the combined mixture on chemibeads (method 1). The other is first coupling with four kinds of chemibeads and combining them as mixture chemibeads (method 2). Coating method 1 is simpler than method 2 in production. Egg white allergen components are clear, including ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4).[25] Compared with egg natural extracts, the application of mixed four components can avert cross-reactivity and distinguish genuine egg white allergy.[25] The result showed that chemibeads of method 1 had a better performance in detecting low-level egg white-sIgE than method 2. In addition, chemibeads of method 1 showed a lower background signal than method 2. In short, chemibeads of method 1 were chosen for subsequent experiments.

Protein modification occurs during the immobilization process when coupling with egg allergens to chemibeads.[26] The allergen epitopes may be occluded because of the binding to the aldehyde group on the surface of chemibeads. However, these epitopes may be exposed in the suitable buffer system, and the absence of steric

TABLE 1 Repeatability and intermediate precision of LICA

Serum pool	Repeatability			Within-run precision		
	Average measured value (kU_A/L)	SD (kU_A/L)	CV (%)	Average measured value (kU_A/L)	SD (kU_A/L)	CV (%)
Low	0.275	0.020	7.29	0.267	0.023	8.64
Middle	1.015	0.028	2.72	1.007	0.050	4.93
High	2.608	0.113	4.34	2.586	0.151	5.85

Abbreviations: CV, coefficient of variation; LICA, light-initiated chemiluminescence assay; SD, standard deviation.

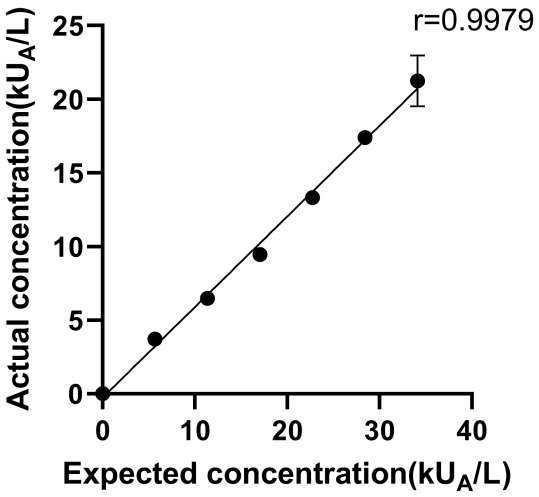

FIGURE 5 Linearity of the assay

hindrance between allergen and antibodies may cause an increase in binding capacity.[27] Based on this theory, seven buffer systems were selected to investigate the best buffer system. According to the data, the serum pools in PB had the highest S/N ratio among seven buffer systems, and the background signal was lower than others. PB was supposed to be the best buffer system, and the optimal condition was 0.01 M PB (pH 7.4) and 0.01% (v/v) Tween 20.

In this assay, we evaluated the performance of LICA for the detection of egg white-sIgE. Precision is defined as the closeness of agreement between independent measurement results obtained under stipulated conditions.[19] It is evaluated by repeatability and intermediate precision. For the repeatability, the CV values ranged from 2.72% to 7.29%. For the intermediate precision, the CV values ranged from 4.93% to 8.64%. Both were statistically acceptable in clinical (CV ≤ 15%). According to the CLSI EP17-A,[21] LoB is defined as the highest measurement result that is likely to be observed for a blank sample. LoD is defined as the lowest amount of analyte in a sample that can be detected with (stated) probability, although perhaps not quantified as an exact value. LoQ is defined as the lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness. The LoB, LoD, and LoQ were 0.000 kUA/L, 0.053 kUA/L, and 0.076 kUA/L. The cut-off value of the ImmunoCAP is 0.35 kU_A/L.[28] The LoQ was lower than the cut-off value and met the clinical requirements. The linearity was defined as the ability to provide results that are directly proportional to the concentration of the analyte in the test sample. The linear range of this method was 0.076–34.125 kU_A/L ($r = 0.9979 \geq 0.9900$), which showed good linearity.

TABLE 2 Method comparison of LICA and ELISA (HOB, China) for detection of egg white-sIgE

LICA	ELISA		Total
	Positive	Negative	
Positive	64	4	68
Negative	4	31	35
Total	68	35	103

Abbreviations: ELISA, enzyme-linked immunosorbent assay; LICA, light-initiated chemiluminescence assay.

It is important to mention that both allergen coupling methods of chemibeads are viable. The antigens can be coupled individually if their coupling conditions are significantly different because of antigen properties. Method 2 allows the multi-detection of antigens with different properties simultaneously. It has a reference value for detecting other significative combinations of allergen components in future.

5 | CONCLUSION

In this study, we established a LICA method for detecting egg white-sIgE rapidly. The application of allergen components can effectively avoid the occurrence of cross-reactivity such as chicken. Performance was evaluated and met clinical laboratory standards. This method is more convenient and faster for the detection of egg white-sIgE. It can better serve the clinical laboratory and is helpful for the diagnosis of egg allergy.

AUTHOR CONTRIBUTIONS

Xin Tan, Huiqiang Li, and Xue Li contributed to conception and design; Lisheng Zheng and Hongbin Shi contributed to provision of study materials or patients; Xin Tan and Dandan Liu contributed to collection and assembly of data; Xin Tan and Bei Zhang contributed to data analysis and interpretation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT TO PARTICIPATE

Informed consent was obtained from all patients.

CONSENT FOR PUBLICATION

All authors read and approved the final work for publication.

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