

# Quantitative Detection of House Dust Mites–Specific IgE by Light-Initiated Chemiluminescence Assay

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**Key words:** inhalant allergens, HDM-specific IgE, light-initiated chemiluminescence assay, quantitative detection, performance, comparison

**Abbreviations:** WAO, World Allergy Organization; HDMs, house dust mites; SPT, skin-prick testing; FEIA, fluorescent enzyme immunoassay; LiCA, light-initiated chemiluminescence assay; CBS, carbonate buffer solution; CL, chemiluminescence; CLSI, Clinical and Laboratory Standards Institute; LoB, limit of blank; LoD, limit of detection; LoQ, limit of quantitation; Pct<sub>B</sub>, percentile of the control specimen result distribution; RLU, relative luminescence unit; R<sub>2</sub>, determination coefficient

*Laboratory Medicine* 2024;55:27–33; <https://doi.org/10.1093/labmed/lmad024>

## ABSTRACT

**Objective:** To establish a new method for quantitative detection of house dust mite (HDM)–sIgE based on light-initiated chemiluminescence assay (LiCA).

**Methods:** The assay was established after optimizing the reaction conditions, and the assay performance was evaluated according to the clinical guidelines. Further, the results of LiCA were compared with those from the ELISA and ImmunoCAP methods.

**Results:** Coefficients of variation for repeatability ranged from 4.22% to 7.69%, and intermediate precision from 8.38% to 10.34%. The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were 0.066 kU<sub>A</sub>/L, 0.165 kU<sub>A</sub>/L, and 0.171 kU<sub>A</sub>/L, respectively. The coefficient of correlation (*r*) between the results of LiCA and ELISA was 0.9263, and the *r* between the results of LiCA and ImmunoCAP was 0.8870.

**Conclusions:** A HDM-sIgE quantitation assay based on LiCA was established, which could be used as a new reliable analytical tool for the determination of HDM-sIgE.

With the advancement of industrialization, the prevalence of allergic diseases is increasing worldwide, especially in newly industrialized countries such as China and India.<sup>1</sup> The World Allergy Organization (WAO) anaphylaxis guidelines show that 40% of the global population is affected by allergic diseases.<sup>2</sup> These diseases not only affect quality of life but also impose a huge economic burden on society.

The existence of allergens is a necessary factor for the development of allergic diseases.<sup>3</sup> Common allergens include 2 main categories: inhalant allergens and food allergens. Dust mites are ubiquitous in our living environment and are one of the most common inhalant allergens.<sup>4,5</sup> The major allergenic dust mites include house dust mites (HDMs) and *Dermatophagoides farinae* in China.<sup>6</sup> More than 50% of patients with allergies are reported to have allergic symptoms to HDMs, and as many as 85% of children with allergic asthma are sensitive to HDMs.<sup>7,8</sup> Early diagnosis of patients with allergies is important to avoid allergens and guide clinical treatment.

Skin-prick testing (SPT) and serum allergen-sIgE detection are the main methods for diagnosing patients who are allergic to HDMs.<sup>9</sup> The serum allergen-sIgE test is widely used due to its safety, simplicity, and convenience.<sup>10</sup> The ImmunoCAP (Thermo Fisher Scientific) system is internationally recognized as the criterion standard for the detection of singleplex allergen-sIgE, which is a fluorescent enzyme immunoassay (FEIA) method with high sensitivity and specificity.<sup>11,12</sup> However, it is not widely used in China because of its high cost and long turnaround period.<sup>13</sup>

Light-initiated chemiluminescence assay (LiCA) is a homogeneous luminescence immunoassay based on singlet oxygen transmission.<sup>14</sup> The method is sensitive, time-saving, wash-free, and simple to operate, all of which make it a promising alternative to existing conventional methods.<sup>15</sup> The results of previous studies<sup>16–18</sup> have successfully established LiCA for the detection of food allergen-sIgE, such as egg white-sIgE and milk protein-sIgE. After that, our team set out to investigate the feasibility of detecting inhalant allergen-sIgE and successfully established LiCA for the quantitative detection of mugwort-sIgE.<sup>19</sup> So far, there have been no reports on LiCA-based assay for other inhalant allergen-sIgE except for mugwort. Consequently, we took HDMs as an example for exploring the feasibility of a LiCA-based method for the quantitative detection of inhalant allergen-sIgE. Also, we validated the performance of this method according to the clinical guidelines to confirm whether it meets the requirements of clinical laboratories.

## Materials and Methods

### Specimens

A total of 108 serum specimens were collected from Tianjin Children's Hospital, China, from March 2022 through August 2022. A total of 90 specimens were collected from patients with HDM allergies, and 18 specimens were obtained from volunteers without HDM allergies. All serum specimens were stored at  $-20^{\circ}\text{C}$  before testing. A total of 85 serum specimens were detected via a capture ELISA kit, and 23 serum specimens were tested by ImmunoCAP. Serum specimens with HDM-sIgE values  $\geq 0.35 \text{ kU}_A/\text{L}$  were regarded as having tested positive. In contrast, serum specimens with HDM-sIgE values  $< 0.35 \text{ kU}_A/\text{L}$  were considered as having tested negative. Informed oral consent was obtained from all patients. The study procedures were designed and carried out based on the Declaration of Helsinki and were approved by the Ethics Committee of the Tianjin Children's Hospital.

### Reagents and Chemicals

We purchased HDM extract from Stallergenes Greer, biotinylated goat anti-human IgE antibody from Thermo Fisher Scientific, human hemoglobin from Merck, and ProClin 300 (Merck) and bilirubin from Solarbio. Naked chemibeads and streptavidin-coated sensibeads were acquired from Beyond Biotech.

### Coupling of HDM Allergens to Chemibeads

HDM allergens were coupled with chemibeads according to a standard procedure described previously.<sup>16</sup> Briefly, the chemibeads were pretreated in carbonate buffer solution (CBS) by sonication. PBS-solubilized HDM allergens were added to CBS-suspended chemibeads. The mixture was incubated overnight at  $37^{\circ}\text{C}$  with slow stirring. The next day,  $8 \text{ mg/mL}$   $\text{NaBH}_4$  and  $75 \text{ mg/mL}$  glycine were added sequentially to the mixture, and the reaction mixture was rotated at room temperature. After washing, the HDM allergens-coated chemibeads were resuspended at  $10 \text{ mg/mL}$  and stored at  $4^{\circ}\text{C}$ .

### Assay Principle and Procedure

The principle of LiCA for detecting HDM-sIgE was shown in [Supplementary Figure 1](#). The entire measurement procedure lasted 45 minutes. First,  $25 \mu\text{L}$  of HDM allergens-coated chemibeads,  $25 \mu\text{L}$  of serum specimens or calibrators, and  $25 \mu\text{L}$  of biotinylated goat anti-human IgE antibody were added to a 96-well microplate. Then, we placed the microplate in a chemiluminescence analyzer (Beyond Biotech) at  $37^{\circ}\text{C}$  for 30 minutes. Next,  $150 \mu\text{L}$  of streptavidin-coated sensibeads were added in the dark. Then the microplate was put back into the instrument and incubated in the dark at  $37^{\circ}\text{C}$  for 15 more minutes. Finally, the chemiluminescence (CL) signals were read by the instrument at  $612 \text{ nm}$ .

### Optimization of the Reaction Conditions

The reaction system of LiCA was influenced by several factors, including the mass ratio of HDM allergens-coated chemibeads, the working concentration of the chemibeads, and the reaction buffer system. First, 3 mass ratios (20:4, 20:8, 20:16) of chemibeads were prepared. Afterward, 3 serum pools containing a high, medium, and low level of HDM-sIgE, respectively, were selected. The serum specimens from the individuals without allergies were used as negative controls. The CL signal of each

specimen was detected by LiCA, and the signal-to-noise (S/N) ratio was calculated to obtain the optimal mass ratio. The selection of serum pools and the determination of optimal conditions in the following experiments were performed in the same manner.

Next, the optimal working concentration of the chemibeads was selected by diluting them to  $12.5 \mu\text{g/mL}$ ,  $25.0 \mu\text{g/mL}$ , and  $50.0 \mu\text{g/mL}$ . To optimize the buffer system, 4 different kinds of buffer systems, including PBS, CBS, HEPES buffer, and Tris-HCl buffer, were used in LiCA to examine all the specimens.

The pH value and concentration of HEPES buffer were further optimized because the HEPES buffer was selected as the best buffer system. Three pH values of 6.7, 7.2, and 7.7 of HEPES buffer were prepared to obtain the optimum pH value, and 3 concentrations of 0.01 M, 0.025 M, and 0.05 M of HEPES buffer were made to obtain the optimal concentration.

Surfactants contribute to opening the disulphide bonds of proteins, which expose the linear epitopes.<sup>20</sup> Therefore, 3 surfactants containing sodium deoxycholate, Triton X100, and Tween 20 were added to the buffer system separately, to explore whether they could improve the S/N ratio. After that, the content of Tween 20 in HEPES buffer was optimized: Tween 20 was used as the best surfactant. Finally, HEPES buffer containing 0.01% Tween 20, 0.05% Tween 20, and 0.1% Tween 20 were prepared to determine the appropriate amount of surfactant.

### Construction of Calibration Curve

Serum specimens with HDM-sIgE concentration of  $35 \text{ kU}_A/\text{L}$  (measured by the ImmunoCAP system) were selected and diluted to  $17.5 \text{ kU}_A/\text{L}$ ,  $3.5 \text{ kU}_A/\text{L}$ ,  $0.7 \text{ kU}_A/\text{L}$ , and  $0.35 \text{ kU}_A/\text{L}$ . Serum specimens from an individual without allergies were used, which measured  $0 \text{ kU}_A/\text{L}$ . All 6 calibration points were detected by LiCA. A calibration curve based on a 4-parameter logistic regression was established using the concentration and the corresponding CL signal, which could be used for quantitative conversion of the detection results.

### Precision

According to Clinical and Laboratory Standards Institute (CLSI) EP5-A2, the precision was evaluated by repeatability and intermediate precision.<sup>21</sup> Three serum pools, containing a high, medium, and low level of HDM-sIgE, respectively, were selected, and each specimen was tested 20 times in an analytical run to evaluate the repeatability. For intermediate precision, the same aforementioned specimens were measured 8 times per day for 5 consecutive days.

### Detection Capability

We tested for the limit of blank (LoB), the limit of detection (LoD), and the limit of quantitation (LoQ), based on clinical guidelines WS/T 514-2017 and CLSI EP17-A.<sup>22,23</sup> To obtain LoB, 4 serum specimens from individuals without allergies, which had each tested negative for allergens, were used as control specimens, and each specimen was measured 5 times per day for 3 consecutive days. Then, 4 serum specimens with HDM-sIgE between 1 LoB and 4 LoB were selected, and each specimen was measured 5 times a day for 3 consecutive days to get the LoD. For the LoQ, a serum specimen ( $0.682 \text{ kU}_A/\text{L}$ ) was diluted to  $0.341 \text{ kU}_A/\text{L}$ ,  $0.171 \text{ kU}_A/\text{L}$ , and  $0.085 \text{ kU}_A/\text{L}$ . Each concentration was tested 20 times in 1 batch.

## Interference Test

The specificity of the established method was assessed by interference tests. According to WS/T 416-2013, a total of 3 serum pools, containing a high, middle, and low level of HDM-sIgE, respectively, were selected.<sup>24</sup> Different concentrations of hemoglobin (2.5 mg/mL, 5 mg/mL, 10 mg/mL), bilirubin (85.5  $\mu$ mol/L, 171  $\mu$ mol/L, 342  $\mu$ mol/L) and biotin (10 ng/mL, 20 ng/mL, 100 ng/mL) were added to the serum specimens as interfering substances. Then, the recoveries were calculated by detecting the concentrations of HDM-sIgE before and after interference.

## Method Comparison

A total of 65 serum specimens were tested by LiCA and commercial ELISA kit (Hob Biotech Group), and the data obtained were analyzed for correlation. Similarly, 23 serum specimens (measured by the ImmunoCAP system) were selected to detect HDM-sIgE by LiCA, and the correlation between the 2 methods was compared.

## Statistical Analysis

The concentration of HDM-sIgE in serum specimens was calculated using ELISACalc (BlueGene Biotech). All statistical analyses were performed using SPSS software, Version 25.0 (SPSS), and  $P < .05$  was considered to be a statistically significant difference. We plotted 4-parameter logistic regression curves and other graphs using GraphPad Prism software, Version 9.0 (GraphPad Software).

## Results

### Establishment of LiCA Method for HDM-sIgE Quantitation

We prepared 3 HDM antigen-coated chemibeads with different mass ratios. The results (FIGURE 1A) showed that when the mass ratio of the chemibeads to antigen was 20:8 and 20:16, the CL signals of the specimens were comparable. FIGURE 1B showed that the S/N ratio was highest when the mass ratio was 20:8. Therefore, we chose 20:8 as the best mass ratio for chemibeads.

Then, we diluted the chemibeads to 12.5  $\mu$ g/mL, 25.0  $\mu$ g/mL, and 50.0  $\mu$ g/mL. The results showed that the S/N ratios of the 3 serum pools were not improved significantly when the concentration was 50.0  $\mu$ g/mL, but the background signals increased (Supplementary Figure 2A

and 2B). Considering the CL signals and S/N ratios, 25.0  $\mu$ g/mL was selected as the most suitable concentration of the chemibeads.

As shown in FIGURE 2A, the S/N ratio was highest when using the HEPES buffer. Therefore, we selected the HEPES buffer as the best buffer system for the subsequent experiments. After that, the pH value of the HEPES buffer was optimized. The results showed that the S/N ratio of a low positive serum pool was the highest when pH was 7.2, and the S/N ratio of a high positive serum pool was the highest when pH was 7.7 (FIGURE 2B). From the perspective of improving sensitivity, 7.2 was used as the optimum pH value of the HEPES buffer. Then, we prepared 0.01 M, 0.025 M, and 0.05 M HEPES buffer. FIGURE 2C shows that the S/N ratio of 0.01 M HEPES buffer was higher than that of the other values. Finally, we chose 0.01 M HEPES buffer with pH 7.2 as the optimal reaction buffer for the LiCA detection system.

The addition of Tween 20 and sodium deoxycholate significantly increased the S/N values of high- and medium-positive serum pools. However, sodium deoxycholate did not improve the S/N values of the low-positive serum pool (FIGURE 2D). Therefore, Tween 20 was chosen as the best surfactant. The results showed that the highest S/N values were obtained for all samples when 0.01% Tween was added to the reaction buffer (FIGURE 2E), which was selected as the optimum content of surfactant in the buffer. FIGURE 3 showed the calibration curve for quantitative detection HDM-sIgE by LiCA. The curve equation was  $y = 9717.05/[1 + (x/31.47)^{-0.89}] + 299.41$ .

### Evaluation of Clinical Performance

To evaluate precision, 3 serum pools were detected by LiCA and then the mean, SD, and CV were calculated. As shown in TABLE 1, in the repeatability study results, the CV ranged from 4.22% through 7.69%. In the intermediate precision study, the CV ranged from 8.38% through 10.34%. No matter the repeatability or intermediate precision, the CV value was less than 15%, which was considered acceptable.

The obtained LoB data were non-Gaussian distributed, and then the 60 data points were ranked to calculate the percentile of the control-specimen result distribution ( $Pct_B$ ). It was required that  $\alpha = 0.05$ ,  $Pct_B = 1 - \alpha = 0.95$ , and ranked position =  $0.5 + (N_B \times Pct_B)$ , where  $N_B$  was the total number of tests, and the arrangement of 60 test results was 57.5. Therefore,  $LoB = X_{57} + 0.5(X_{58} - X_{57})$ . In this study,  $X_{57} = 0.056$ , and  $X_{58} = 0.075$ , so LoB was 0.066 kU<sub>A</sub>/L.

**FIGURE 1.** Optimization of mass ratio of chemibeads coated with antigen. A, Effect of chemibeads mass ratios on chemiluminescence (CL) signal. B, Effect of chemibeads mass ratios on the signal-to-noise (S/N) ratio. RLU, relative luminescence unit.

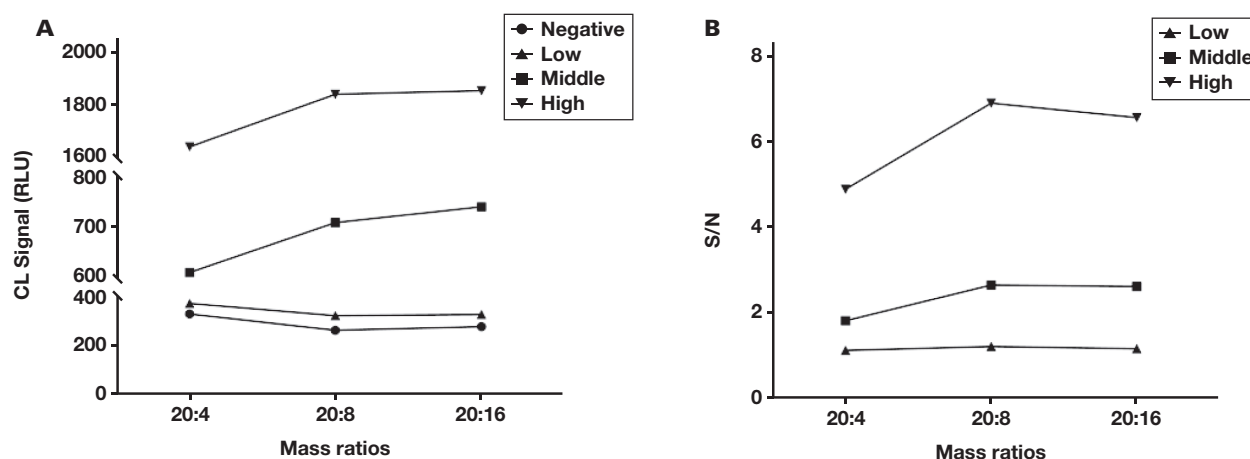


**FIGURE 2.** Optimization of reaction buffer system and surfactant. A, Comparison of the signal-to-noise (S/N) ratio of 3 serum pools in 4 reaction buffer systems. B, Comparison of S/N ratio of 3 serum pools in HEPES buffer with 3 pH values. C, Comparison of S/N ratio of 3 serum pools in HEPES buffer (pH, 7.2) with 3 concentrations. D, Comparison of S/N ratio of 3 serum pools in HEPES buffer (0.01 M; pH, 7.2) with 3 surfactants. E, Comparison of S/N ratio of 3 serum pools in Tween 20 with 3 additional specimens.

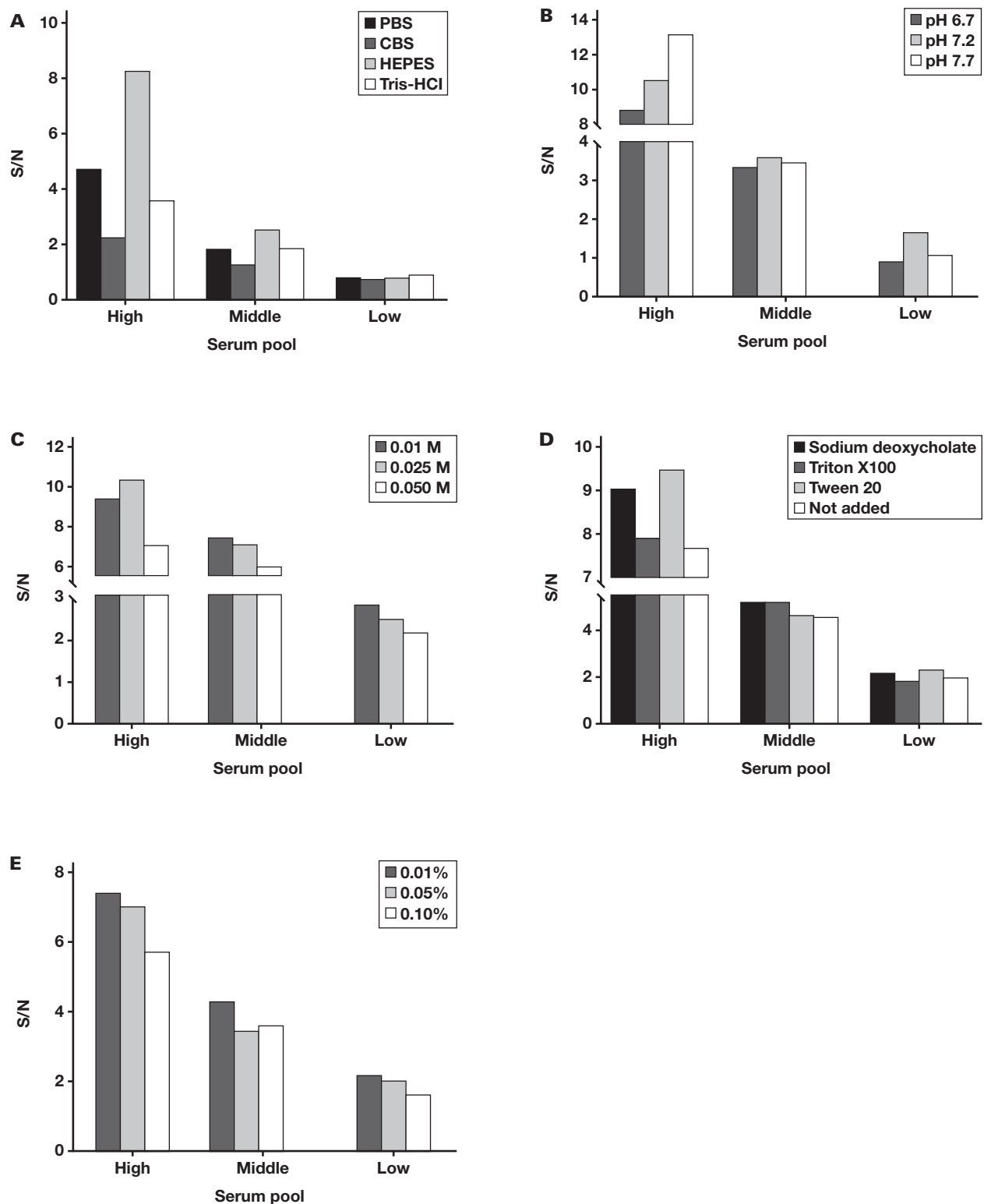


**FIGURE 3.** Calibration curve of light-initiated chemiluminescence (CL) assay for detecting house dust mite-sIgE ( $R^2 = 1.000$ ).

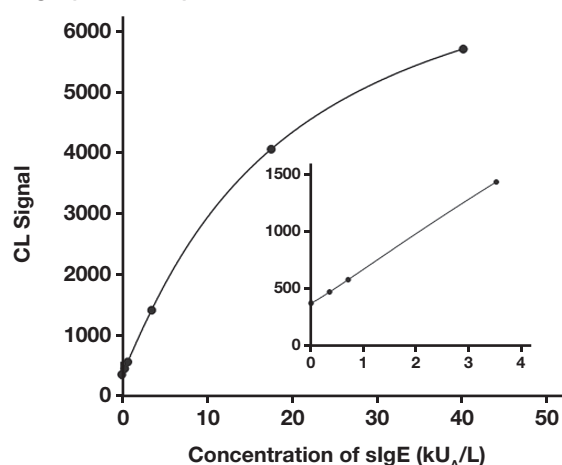


**TABLE 1.** Repeatability and Intermediate Precision

| Serum Pool | Repeatability                 |       | Intermediate Precision        |       |
|------------|-------------------------------|-------|-------------------------------|-------|
|            | Mean (SD), kU <sub>A</sub> /L | CV, % | Mean (SD), kU <sub>A</sub> /L | CV, % |
| Low        | 0.52 (0.04)                   | 7.69  | 0.58 (0.06)                   | 10.34 |
| Middle     | 3.75 (0.25)                   | 6.67  | 3.70 (0.31)                   | 8.38  |
| High       | 7.35 (0.31)                   | 4.22  | 7.75 (0.69)                   | 8.90  |

The LoD data showed Gaussian distribution. Therefore, it was calculated according to the following formula:

$$\text{LoD} = \text{LoB} + \frac{1.645}{1 - \frac{1}{4(N-K)}} \times \text{SD}_s$$

for this study,  $N = 60$ ,  $K = 4$ , and  $\text{SD}_s = 0.06$ , so the LoD of the method was 0.165 kU<sub>A</sub>/L.

For the LoQ, the CV value was required to be less than 15% and not lower than LoD, so the LoQ of this method was 0.171 kU<sub>A</sub>/L.

The specificity of the established method was evaluated by interference testing. As shown in [Supplementary Table 1](#), the recoveries ranged from 80% to 120% when the specimens contained less than 5 mg/mL hemoglobin, less than 342 μmol/L bilirubin, and less than 10 ng/mL biotin.

## Methods Comparison

The results showed that the detection method established in this study correlated well with ELISA and ImmunoCAP. The regression equations and correlation coefficients were as follows:  $y = 0.8646x + 0.2607$ ,  $r = 0.9263$  and  $y = 0.2279x + 0.2286$ ,  $r = 0.8870$  ([FIGURE 4A](#) and [4B](#)).

As shown in [FIGURE 4C](#), the Bland-Altman test was applied to analyze the difference between LiCA and ELISA. The results showed that the mean deviation between LiCA and ELISA was 0.1. Mean ( $\pm 1.96$  SD) was used as the 95% consistency limit. Thus, the lower and upper limits for the 2 methods were -2.6 and 2.8, respectively. The number of data exceeding the lower and upper limits was 5, which means that 92.31% of the data distribution was within the acceptable range. Therefore, there

was good agreement between LiCA and ELISA, and the assay results were reliable. The same method was used to analyze the agreement between LiCA and ImmunoCAP ([FIGURE 4D](#)). The results showed that 95.65% of the data were distributed within the acceptable range. Thus, the 2 methods were correlated.

## Discussion

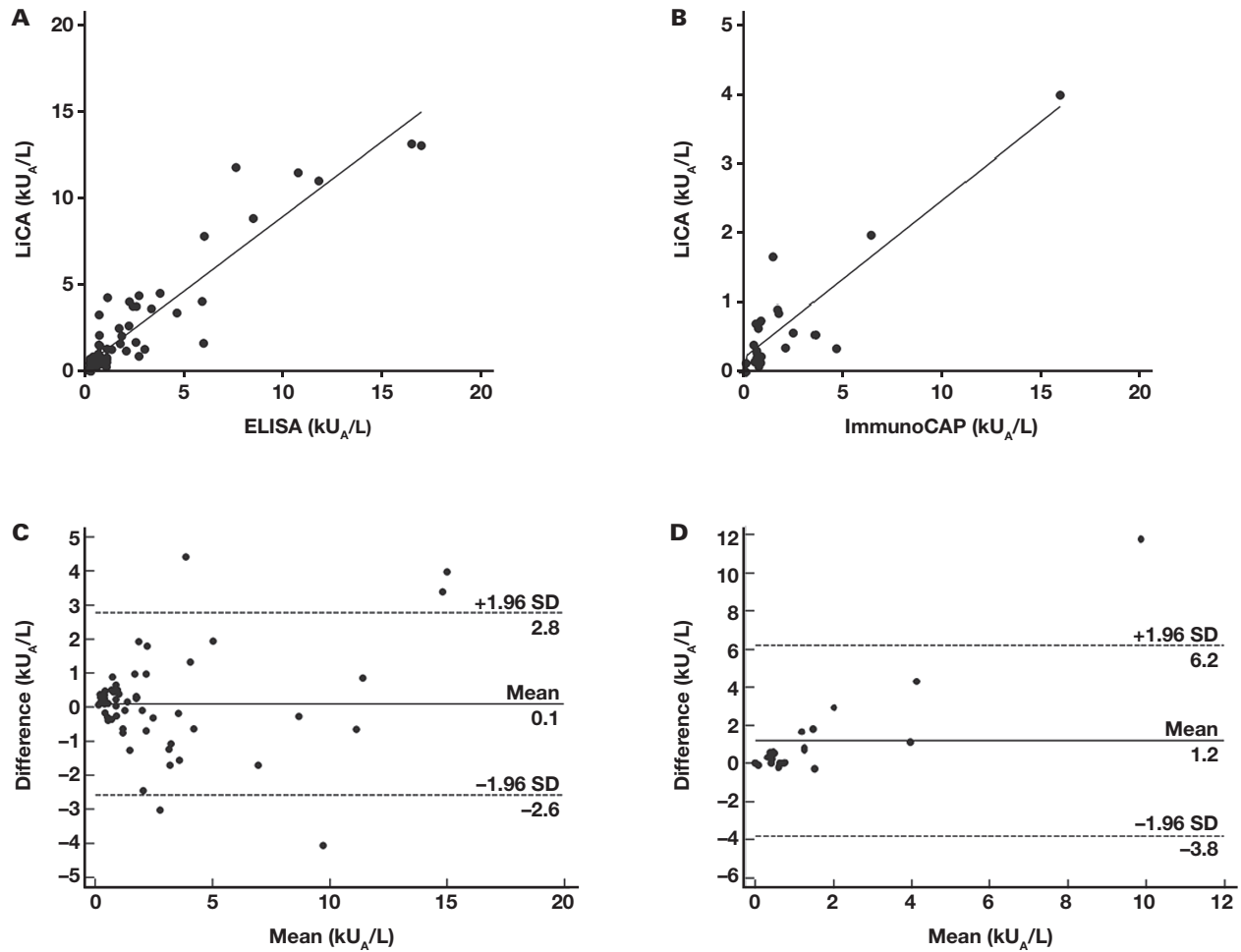
LiCA is a homogeneous immunoassay based on nanoparticles, which originated from the lumino-oxygen channel immunoassay in the 1990s.<sup>14</sup> The method does not require washing steps, and the process can be completed within 1 hour.<sup>25</sup> The results of previous studies, such as Zhang et al.,<sup>18</sup> have revealed that the chemibeads can provide more binding sites for allergen proteins due to their larger specific surface area, so allergen-specific IgG interference can be significantly reduced. Moreover, the reagents are simple to prepare: allergen-coated chemibeads have mature standard procedures, and biotinylated anti-human IgE antibodies and streptavidin-coated sensibeads (universal solution) are commercially available. Thus, the method is easy to commercialize and automate with domestic equipment. These advantages make LiCA suitable for allergen-sIgE detection. Therefore, a quantitative LiCA-based assay was established in this study for the detection of HDM-sIgE.

For HDM-sIgE detection, there are 2 analysis modes to select, namely the capture secondary antibody mode or the solid-phase antigen mode. The former is called the capture method; the latter is called the indirect method. In this study, the indirect LiCA analysis mode was applied. The targeting antibody preferentially binds to the solid-phase antigen, which can reduce the interference of nontarget antibodies. HDM allergens are coupled to chemibeads by chemical bonding, and allergen epitopes may be masked due to binding to aldehyde groups on the surface of the chemibeads.<sup>26,27</sup> However, these masked epitopes may be exposed in a buffer system of suitable pH and ionic strength, so 4 commonly used buffer systems were prepared for the study. Based on the experimental results, 0.01 M HEPES (pH 7.2) was chosen as the best buffer system, and 0.01% Tween 20 was added to it.

As a homogeneous immunoassay, LiCA does not require separation or multiple washing steps, which eliminates the effect of washing errors. According to CLSI EP5-A2, precision is verified by repeatability and intermediate precision.<sup>21</sup> The results showed that the CV values for repeatability were 4.22%, 6.67%, and 7.69% and for intermediate precision were 8.38%, 8.90%, and 10.34%. Both were acceptable in clinical practice ( $<15\%$ ). Only 2.50 μL of serum was used for each test, which is significantly lower than the 50 μL used by the ImmunoCAP system (50 μL).<sup>28</sup> Therefore, the method is suitable for specimens from infants and children. Moreover, LiCA shows excellent sensitivity because each sensibead can release 60,000 singly linear oxygen ions per second for signal amplification.<sup>29</sup>

The sIgE cut-off value for allergic sensitization determined by the ImmunoCAP system is 0.35 kU<sub>A</sub>/L, which is derived from the detection limit of the first assay available.<sup>30</sup> However, the results of some research<sup>31</sup> have shown that the decision based on the clinical decision limit was in disagreement with the SPT. This may be partially attributed to the detection limit of the ImmunoCAP, which misses some specimens that should test positive. Moreover, this sIgE cut-off value is not suitable for all allergens. In our research findings, the LoD and LoQ of the LiCA method were 0.165 kU<sub>A</sub>/L and 0.171 kU<sub>A</sub>/L, respectively. Both 0.165 kU<sub>A</sub>/L and 0.171 kU<sub>A</sub>/L were lower than 0.35 kU<sub>A</sub>/L, implying that the

**FIGURE 4.** Methods comparison. A, Correlation analysis between light-initiated chemiluminescence assay (LiCA) and ELISA ( $y = 0.8646x + 0.2607$ ;  $r = 0.9263$ ). B, Correlation analysis between LiCA and ImmunoCAP ( $y = 0.2279x + 0.2286$ ;  $r = 0.8870$ ). ImmunoCAP is manufactured by Thermo Fisher Scientific Inc. C, Bland-Altman analysis between LiCA and ELISA. D, Bland-Altman analysis between LiCA and ImmunoCAP.



established method may be better in differentiating the specimens with positive results that have low concentrations of antibodies.

Because LiCA does not require multiple washing steps, it may be affected by interfering components in the serum specimens. Therefore, we chose hemoglobin, bilirubin, and biotin as interfering substances to evaluate the anti-interference ability of the method. The data showed that the method could tolerate 5 mg/mL bilirubin and 342  $\mu$ mol/L hemoglobin in serum specimens. Further, physiological concentrations of biotin in serum had no significant effect on the results. However, increasing the concentration of biotin resulted in a significant decrease in CL signal. Moreover, LiCA showed a statistically significant correlation with ELISA ( $r = 0.9263$ ;  $P < .001$ ) and ImmunoCAP ( $r = 0.8870$ ;  $P < .001$ ). Bland-Altman plotting demonstrated acceptable agreement between LiCA and the 2 other methods for detecting HDM-sIgE, indicating its potential clinical application in the detection of HDM-sIgE.

This study had certain limitations. First, different modes of detection (capture mode for ELISA and indirect mode for LiCA) and inconsistent results may be observed between the 2 analytical systems. Second, the small differences in HDM-sIgE levels for several specimens may be due to different allergen components, which need to be demonstrated by further studies.

In conclusion, we have successfully established an indirect LiCA for the quantitative detection of HDM-sIgE in serum. This new method improves on the shortcomings of the traditional methods, which tend to take a long time and tend to be complicated to perform. Also, the method had a good performance and met clinical laboratory standards. As a homogeneous immunoassay, there are no separation and washing steps. Therefore, LiCA can be used as a new rapid, convenient, and efficient method for HDM-sIgE quantification in medical clinics.

### Supplementary Data

Supplemental figures and tables can be found in the online version of this article at [www.labmedicine.com](http://www.labmedicine.com).

### Acknowledgments

We thank the Clinical Laboratory of Tianjin Children's Hospital, China, for material support. This work was supported by the National Natural Science Foundation of China (81772259).

## Conflict of Interest Disclosure

The authors have nothing to disclose.

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