



Research paper

Development of a sensitive and stable chemiluminescent immunoassay for detection of birch pollen allergic specific IgE based on recombinant Bet v1 protein

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ABSTRACT

Background

Quantitative detection of allergens is of great significance for clarifying the cause, treatment, and prevention of allergy disease. Birch pollen is one of the most common inhalational allergens and Bet v1 is the major component allergen of birch allergen. This study aims to develop a stable and sensitive chemiluminescence immunoassay (CLIA) for the detection of birch pollen allergic specific IgE (sIgE) based on recombinant Bet v1 (rBet v1) protein.

Methods

rBet v1 protein was expressed in *Escherichia coli* and purified. Then rBet v1 was applied to detect sIgE in human serum. The performance of the established CLIA was evaluated and compared with Phadia rBet v1 fluorescence enzyme immunoassay (FEIA) system.

Results

The developed CLIA for sIgE to rBet v1 detection shows excellent performance. The assay showed a linear range from 0.1 to 100 IU/mL, with a low detection limit of 0.06 IU/mL. A total of 164 samples were evaluated by CLIA and compared with the results of FEIA. The positive, negative, and total coincidence rate was 90.6% (87/96), 91.2% (62/68), and 90.9% (149/164), respectively. The r-value of Spearman's rank correlation analysis was 0.935 ($P < 0.001$). The use of high levels of bilirubin (50 mg/dL), hemoglobin (400 mg/dL) and lipid (2000 mg/dL) didn't interfere with the results.

Conclusions

The proposed CLIA exhibits excellent performance for the detection of rBet v1 specific IgE. It can be a reliable tool for the early diagnosis of hypersensitivity.

1. Introduction

Allergy is a serious problem affecting approximately one-quarter of individuals worldwide (Hedlin et al., 2009). As it is common to react to several allergens, multiple allergens may cause multisystem allergic diseases such as asthma, rhinitis, and eczema, which have a great impact on human health. At present, with the increase of the varieties of food, medicine, and diagnostic agents, there are more and more allergens, and the anaphylaxis caused by them are increasing (Karmaus et al., 2018; Tan and Corren, 2011; Zheng et al., 2011). The detection of specific immunoglobulin E (sIgE) produced by exposure to the corresponding allergen is important for the diagnosis, treatment, and prevention of

allergic diseases induced by type I hypersensitivity responses (Park et al., 2018). The diseases associated with IgE-mediated hypersensitivity include asthma, allergic rhinitis, atopic dermatitis, drug allergy, food allergy, and anaphylaxis. The precise identification of the clinically relevant allergen is significant for allergen avoidance and appropriate immunotherapy selection (Cianferoni, 2016; Ansotegui et al., 2020).

Pollen from birch and other trees of *Betulaceae* and *Fagaceae* families is the most dominant tree pollen type found in Northern and Central Europe, which is a major cause of allergic rhinitis and asthma symptoms (Smith et al., 2014; Canova et al., 2013; Caillaud et al., 2015). There are more than 100 million patients who suffer from birch pollen allergies worldwide. In Europe, birch-related clinically sensitization affects

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around 19.6% of the allergic population (Pablos et al., 2016; Biedermann et al., 2019; Migueles et al., 2014). The major allergens from Fagales trees are classified as members of the pathogenesis-related-10 (PR-10) proteins. Bet v1 from birch pollen is a 17 kD protein generally acknowledged as the main sensitizer and marker allergen of this family (Hauser et al., 2011; Sekerková and Poláčková, 2011; Von Loetzen et al., 2014; Reinmuth-Selzle et al., 2014; Goodman and Breiteneder, 2019). Around 95% of birch pollen-sensitized patients have specific IgE antibodies to Bet v1 (Wolthers, 2012). Moreover, Bet v1 shows extensive cross-reactivity with homologous proteins from the related tree pollen and foods such as apple, cherry, and celery (Geroldinger-Simic et al., 2013; Kitzmüller et al., 2019; Kinaciyan et al., 2018; Jahn-Schmid et al., 2005; Popescu, 2015).

In recent years, component resolved diagnosis (CRD) has been a major advance in the diagnosis of allergic diseases and greatly improved diagnostic accuracy in the fields of respiratory and IgE-mediated food allergy (Matricardi et al., 2016; Brusca et al., 2019; San Miguel-Rodríguez et al., 2019). CRD shows the true sensitization components of allergic patients by distinguishing the sensitization of extremely cross-reactive labile molecules (PR-10, Profilin) and helps to anticipate the risk and the guidance of oral allergy syndrome (OAS).

Chemiluminescence immunoassays have been widely applied to routine clinical analysis due to their high sensitivity, wide detection range, methodological reliability, and relative stability (Dong et al., 2016; Wang et al., 2019). However, few reports have been found on the establishment of chemiluminescence immunoassay for the detection of sIgE to Bet v1.

In this study, a highly sensitive and stable chemiluminescence immunoassay was established based on recombinant birch pollen Bet v1 protein. Cost-effective and easy production at a large scale makes *Escherichia coli* (*E. coli*) the first choice of recombinant protein expression programs. In addition, to evaluate the detection performance of chemiluminescence immunoassay for rBet v1, the results were compared with those from the FEIA method (Phadia immunoCAP system).

2. Materials and methods

Restriction enzymes *EcoRI* and *XhoI* were purchased from Thermo Fisher (Waltham, MA, USA). Biotin Conjugation Kit was purchased from Innova Biosciences (Cambridge, UK). Ampicillin (Amp) and isopropyl- β -D-thiogalactoside (IPTG) were purchased from Solarbio Technology Co., Ltd. (Beijing, China). Plasmid extraction kits and competent cells Rosetta were purchased from Tiangen Technology Co., Ltd. (Beijing, China). Ni-NTA Agarose was purchased from GE Healthcare Life Sciences (Uppsala, Sweden). The chemiluminescence detection was carried out using the automatic chemiluminescence analyzer from Rayto Life Science Co., Ltd. (Shenzhen, China).

2.1. Gene synthesis and cloning

Bet v1 cDNA (GenBank: X15877) was synthesized and cloned into the expression vector pET21a + at *EcoRI* and *XhoI* sites from Nanjing Genscript of China. The recombinant expression vector was named as pET21a-Bet v1 and it was subjected to two-step digestion with *XhoI* and *EcoRI* restriction enzymes. After digestion, the product was electrophoresed on low melting 1% agarose gel. After evaluation of the gel, the plasmids were subjected to sequencing (JinWeizhi, Suzhou, China). The resulting construct was transformed into *E. coli* BL21(DE3). pET21a-Bet v1 and *E. coli* strain BL21 (DE3) were stored in our laboratory.

2.2. Protein expression and purification

For recombinant Bet v1 expression, the transformants were grown in LB culture media containing 50 μ g/mL ampicillin until OD₆₀₀ reached 0.6, followed by induction with 0.5 mM IPTG for 15 h at 30 °C. The harvested cells were lysed by cell lysis buffer (20 mM Tris, 500 mM

NaCl, 1% Nonidet P40, 5 mM EDTA, 1 mM PMSF, pH 7.9) and centrifuged at 9500 $\times$ g for 10 min. Recombinant Bet v1 was expressed in an insoluble fraction and accumulated as inclusion bodies. The inclusion bodies were solubilized in inclusion body dissolving buffer (50 mM phosphate buffer containing 8 M urea, 150 mM NaCl, 5 mM imidazole, pH 7.9). The protein solution was then centrifuged at 15,000 $\times$ g for 10 min. The centrifuged supernatant was filtered through a 0.45- μ m membrane and then loaded onto a HisTrap FF 5 mL column. Then the column was washed with 10 column volumes of washing buffer containing 20 mM imidazole. The target protein was eluted using the elution buffer containing 500 mM imidazole. Subsequently, the eluted solution containing target protein was collected and dialyzed against 100 mM phosphate buffer (pH 7.4) with 6 M urea. The purified rBet v1 was analyzed by SDS-PAGE.

2.3. Immunoassay design and test procedure

rBet v1 chemiluminescence immunoassay was developed employing biotinylated rBet v1, streptavidin-labeled nano-magnetic particles, alkaline phosphatase (ALP)-labeled anti-IgE antibody, and 4-methoxy-4-(3-phosphatephenyl)-spiro- (1,2-dioxetane-3,2-adamantane) (AMPPD) as substrate. The assay adopted 6-point calibration with traceable calibrators, and the concentrations were at 0, 0.35, 0.7, 3.5, 17.5, 50, and 100 IU/mL, respectively. The reaction was carried out automatically on the Lumiray 1600 automatic nanometer magnetic particle chemiluminescence detection analyzer. First, 15 μ L of the sample, 45 μ L of streptavidin-labeled nano-magnetic particles, and 15 μ L of biotinylated rBet v1 were mixed and incubated at 37 °C for 15 min. The sIgE to rBet v1 in the sample specifically bound with biotinylated rBet v1 and further attached to the surface of magnetic particles. Excess biotinylated Betv1 allergen was removed through washing. Secondly, 130 μ L of ALP-labeled mouse anti-human IgE secondary antibody was added to form a solid-phase antigen antibody-labeled secondary antibody complex. After washing, unbound enzyme-labeled antibodies and other substances were removed. Finally, 200 μ L of luminescent substrate AMPPD was added. The enzyme on the enzyme-labeled antibody catalyzed the emission of photons by the luminescent substrate. The luminous intensity is directly proportional to the sIgE antibody content in the sample. The calibration curve was fitted with four parameters, and the luminous intensity of the test object was substituted into the calibration curve to obtain the content of sIgE to rBet v1 in the sample.

2.4. Performances of rBet v1 chemiluminescence immunoassay

2.4.1. Calibration curve and limit of detection (LOD)

Six different concentrations of the standard solution were detected to establish the calibration curve. The IgE calibrators are traceable (via an unbroken chain of calibrations) according to the equivalent 3rd International Standard 11/234 of Human Serum Immunoglobulin E from World Health Organization (WHO). This uncertainty (expanded uncertainty; $k = 2$) was calculated in accordance with the "Guide to the expression of uncertainty in measurement" (GUM: 1993) in the traceability chain. The limit of quantitation within an assay is set to ensure that reported low quantitative results are higher than the background signal (noise-chemical, instrument, and from non-specific IgE binding). LOD testing was evaluated according to Global Consensus Standardization for Health Technologies (NCCLS) document EP17-A (Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline guidelines). Calibrator dilution buffer was repeated 20 measurements, then calculated the mean and standard deviation of these measurements, multiplied this SD by a factor of 2, add that to the mean of the replicates, and call this value the LOD. The aim of LOD is lower than 0.1 IU/mL.

2.4.2. Dilutional linearity

Dilutional linearity was evaluated on triplicate of a dilution series of

the sample at 100 IU/mL following the NCCLS document EP6-A. This is a statistical approach for the evaluation of the linearity of quantitative measurement procedures. The sample was diluted by S0 to obtain a series of dilutions of 1/2, 1/5, 1/25, 1/100, 1/500, and 1/1000 that of the expected concentration. Each sample was measured doubly in parallel. The measured values were compared to the expected values by linear regression analysis.

2.4.3. Precision

Precision using quality control materials was evaluated following guideline EP5-A2 (Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition). We measured three different concentrations for 10 times within one assay. Similarly, the controls were analyzed on 10 different days using the protocol (5 replicated per run) to obtain the inter-assay variation. The performance goals with intra-assay and inter-assay CVs are $\leq 8.0\%$ and $\leq 10.0\%$, respectively.

2.4.4. Method comparison

Method comparison testing was constructed by NCCLS document EP9-A2 (Method Comparison and Bias Estimation using patient samples, Approved Guideline, Second Edition). The methodological comparison was used to collect 164 clinical samples. Phadia ImmunoCAP FEIA method is currently the gold-standard method for sIgE detection. The sIgE to rBet v1 content in clinical samples was detected by the sIgE-CLIA and Phadia ImmunoCAP FEIA method simultaneously. The cut-off values of both methods were 0.35 IU/mL. The results were divided according to class determination range, expressed as IU/mL: class 0 (0.00–0.34 IU/mL), class 1 (0.35–0.69 IU/mL), class 2 (0.70–3.49 IU/mL), class 3 (3.50–17.49 IU/mL), class 4 (17.50–49.99 IU/mL), class 5 (50.00–99.99 IU/mL), and class 6 (>100 IU/mL) (Van Hoeyveld et al., 2015).

2.4.5. Interference

Endogenous interference studies were performed according to CLSI EP07-A2. To evaluate the interference effect of Endogenous Substance, bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL) were added into a sample respectively and were analyzed in duplicates using the CLIA-sIgE assay. Compared with the tube samples without additives, the interference deviation was calculated, and the acceptable interference bias is less than 10%.

2.4.6. Cross-reactivity

To evaluate cross-reactivity, 30 samples of carrot allergic, 27 samples of apple allergic, and 30 samples of celery allergic were measured respectively.

2.5. Statistical analysis

The positive (negative) coincidence rates were calculated as follows: the number of both tests showing a positive (negative) result/the number of the gold standard Phadia ImmunoCAP system showing a positive (negative). The total coincidence rates were calculated as follows: (the number of both tests showing a positive result+the number of both tests showing a negative result)/the total number of tests.

Chi-square (χ^2) was used for the consistency of the two methods, and Kappa tests were used for qualitative testing. We assessed and categorized the kappa value as almost perfect (0.8–1.0), substantial (0.6–0.8), moderate (0.4–0.6), fair (0.2–0.4), and poor (below 0.2) (Francesca et al., 2019; Mukaka, 2012). A *P*-value of <0.05 was considered to be significant in both two tests. Spearman's rank correlation analysis was used to evaluate the semi-quantitative correlation between grades 0–6. Both the indices and the coefficients were closer to 1, indicating the values were in good agreement. It showed highly positive correlations between them ($P < 0.001$). Spearman's correlation coefficients were classified into very high positive correlation (0.9–1.0), high positive

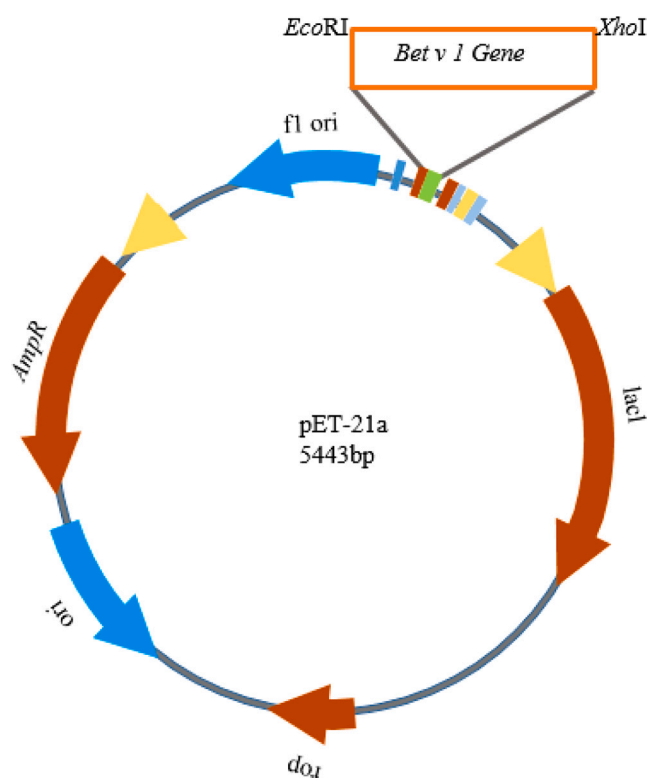


Fig. 1. Schematic of construction of recombinant plasmid Bet v1-pET21a+. The target gene fragment of Bet v1 was cloned into the pET21a+ vector using the *XhoI* and *EcoRI* restriction enzymes.

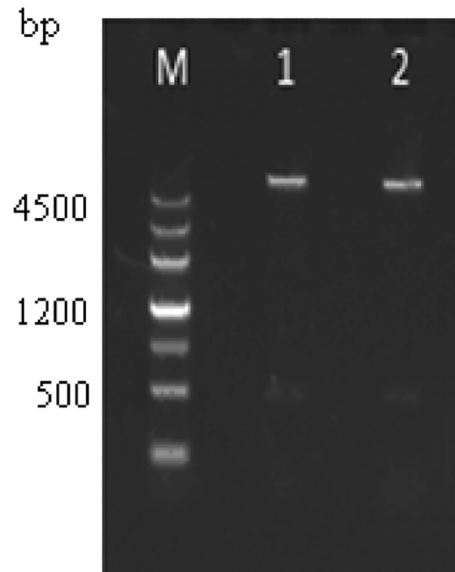


Fig. 2. The resultant plasmids were purified and verified using agarose gel. Lane M shows 7 DNA markers with fragment sizes of 4500, 3000, 2000, 1200, 800, 500, and 200 bp. Lanes 1 and 2 show the recombinant plasmids digested by *XhoI* and *EcoRI* restriction enzymes.

(0.7–0.9), moderate positive (0.5–0.7), low positive (0.3–0.5), or negligible (below 0.3) correlation (Zhang and Qi, 2011). The linear correlation was evaluated between the CLIA and FEIA measurements. All the statistical analysis was performed using the SPSS statistical software (SPSS for Mac, version 21).

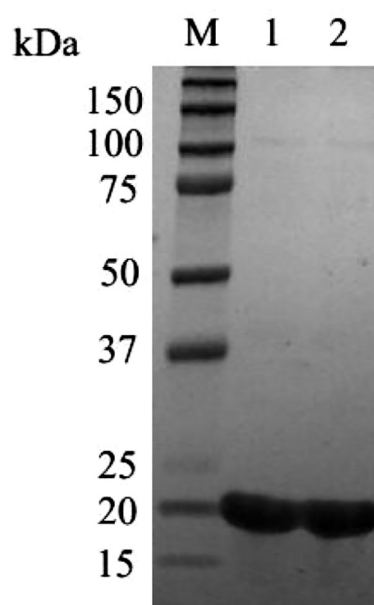


Fig. 3. SDS-PAGE shows the result of rBet v1 expression. Lane M: molecular weight protein markers. Lanes 1 and 2: purified rBet v1.

3. Results

3.1. Restriction enzyme digestion of recombinant plasmid

The synthesized sequence for Bet v 1 gene (Genbank Accession number X15877) was optimized by Genscript (Appendix A). The target gene fragment was cloned into the expression vector pET21a + at *EcoRI* and *XhoI* sites (Fig. 1). The recombinant plasmid pET21a-rBet v1 was digested by two enzymes (*EcoRI* and *XhoI*) and the products were applied to agarose gel electrophoresis. The target gene fragment is about 500 bp, while the vector pET-21a + fragment is about 5000 bp. It shows in the electropherogram that there are two separate DNA fragments, the sizes are the same as the theoretical ones (Fig. 2). The analysis Sequence confirmed that the gene sequence of the expression vector is consistent with the design. The cDNA for the major allergen of the birch pollen, rBet v1, was successfully cloned by sequencing.

3.2. Identification of recombinant Bet v1 protein

The recombinant Bet v1 was expressed in the form of insoluble components and accumulated as inclusion bodies. The inclusion bodies were solubilized with 8 M urea, then the target protein was affinity purified by HisTrap FF 5 mL column. SDS-PAGE analysis exhibits a

Table 1

The value and uncertainty of calibrators.

	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6
Calibrator value (IU/mL)	0.348	0.703	3.515	17.551	50.657	99.864
Uncertainty (IU/mL)	0.014	0.025	0.130	0.646	1.905	3.835

cal: calibrator.

single band with the predicted size near 20 kDa, representing the target protein (Fig. 3). Purification of rBet v1 was successfully done, with purity higher than 90%.

3.3. Performances of the CLIA-sIgE assay

3.3.1. Principle of the CLIA

A chemiluminescence immunoassay based on recombinant Bet v1 protein was established and evaluated. The sIgE to rBet v1 in the sample specifically binds to biotinylated rBet v1 and attaches to the surface of magnetic particles. Excess biotinylated Betv1 allergen is removed through washing. Then alkaline phosphatase-labeled (ALP) mouse anti-human IgE secondary antibody is added to form a solid-phase antigen antibody-labeled secondary antibody complex. After washing, unbound enzyme-labeled antibodies and other substances are removed. Finally, luminescent substrate AMPPD is added. ALP on the enzyme-labeled antibody catalyzes the emission of photons by the luminescent substrate. The luminous intensity is directly proportional to the sIgE antibody content in the sample (Fig. 4).

3.3.2. Calibration curve and limit of detection (LOD)

The permanent measurement procedure evaluates the product calibrators (Table 1). The standard curve was plotted using a Four Parameter Logistic (4-PL) curve-fit. The regression equation is $y = (8016.78 - 52,335,973.44)/(1 + (x/1063.82)^{0.98}) + 52,335,973.44$, dose as independent variable x (IU/mL), response as dependent variable y (RLU), the r -value is 1.0000 (Fig. 5). Under the optimal conditions, the dose-response curve was measured with the CLIA method. The detection limit is calculated by taking the mean RLUs signal (S_0 , 20 replicates) at zero concentration plus two times the standard deviation ($S_0 + 2SD$) into the dose-response curve, and the obtained sIgE to rBet v1 detection limit is 0.06 IU/mL (Table 2).

3.3.3. Dilutional linearity

The linear regression equation was mapped with the expected values as the X-axis and the measured values as the Y-axis. The linear regression equation is $y = 0.991x + 0.0647$. The relationship between the measured concentrations and the expected concentration of the diluted sIgE to rBet v1 shows a good linear correlation coefficient of 0.9998 (Fig. 6). The CLIA-sIgE assay shows a wide linear range from 0.1 to 100

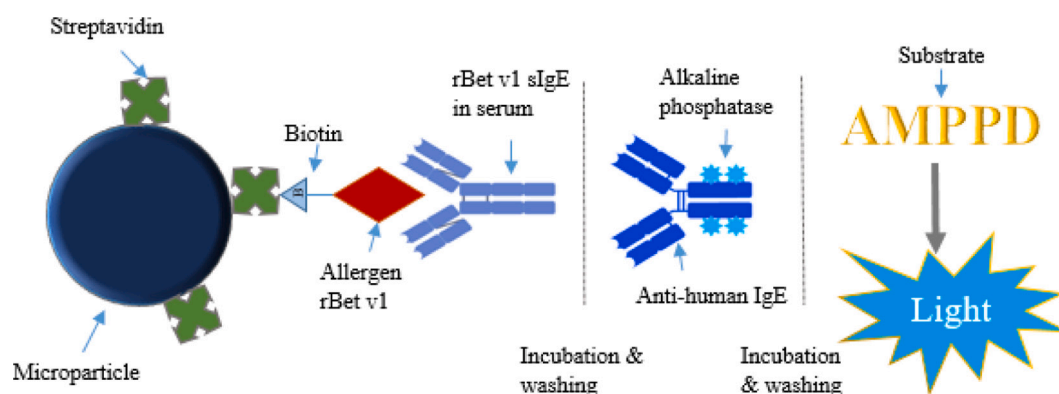


Fig. 4. Schematic diagram of the CLIA-sIgE assay.

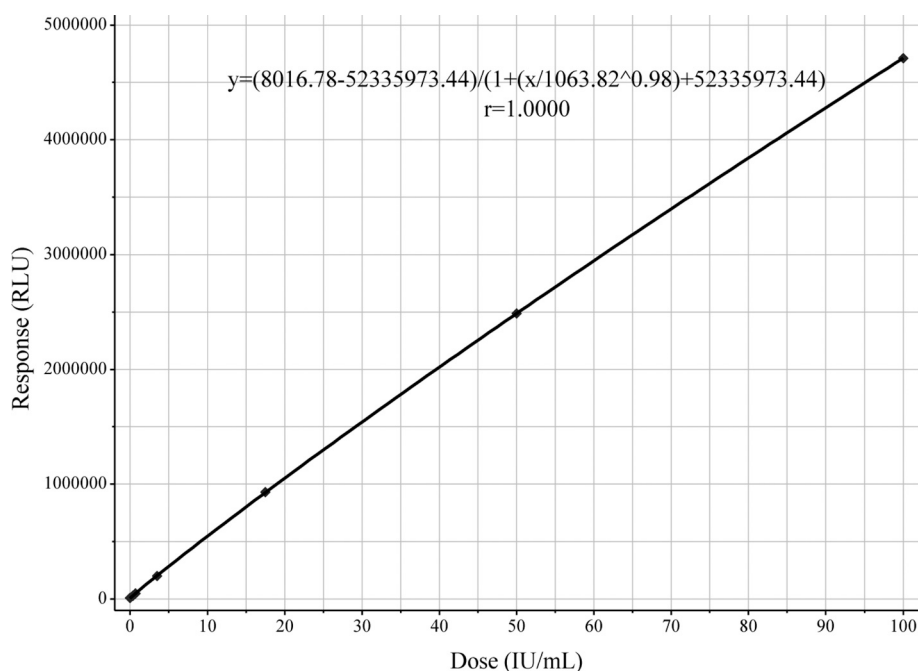


Fig. 5. The dose-response curve.

Table 2
LOD for the CLIA-sIgE assay.

Sample	M (RLU)	SD (RLU)	M + 2SD(RLU)	LOD (IU/mL)
S0	10,969	280	11,530	0.06

IU/mL.

3.3.4. Precision

The precision reflects the accuracy and repeatability of the CLIA method. Three different concentrations of quality control serum (high, middle, and low) were tested to evaluate the intra-assay and inter-assay CVs. The intra-assay variation was evaluated by analyzing each concentration ten times per run in 1 day. Similarly, these samples were

analyzed on 5 different days using the same protocol (5 replicates per run) to obtain the inter-assay variation. As shown in Table 3, intra-assay and inter-assay CVs are <8.0% and <10.0%, respectively.

3.3.5. Method comparison

The positive, negative, and total coincidence rate are 90.6% (87/96), 91.2% (62/68), and 90.9% (149/164) compared with the Phadia fluorescence system, respectively. High concordance is found between qualitative sIgE to rBet v1 results obtained by CLIA and Phadia ImmunoCAP system as reflected by all coincidence rate >90.0%. The χ^2 value is 108.494, k-value is 0.813 (>0.75). *P*-values calculated by Pearson's chi-square and Cohen's kappa analysis are all <0.05. All results indicate an excellent agreement (Table 4).

Classification correlation analysis using Spearman's correlation coefficients is shown in Fig. 7. Spearman's rank correlation analysis

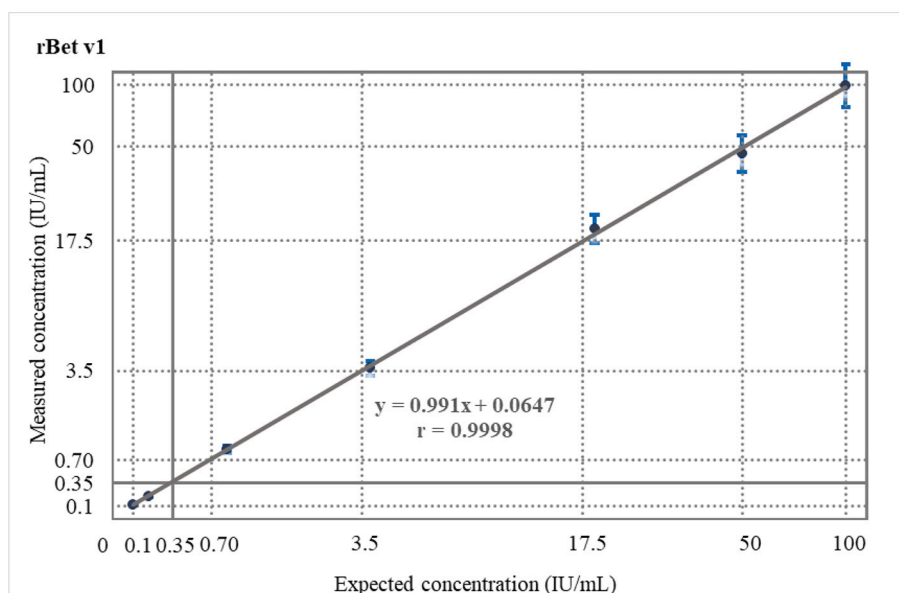


Fig. 6. Dilutional linearity evaluated by a series of diluted samples range from 0.1 IU/mL to 100 IU/mL.

Table 3

Intra-assay and inter-assay variability for the CLIA-sIgE assay.

Sample no.	Intra-assay			Inter-assay		
	Times of replication	Concentration (IU/mL)	CV (%)	Days of replication	Concentration (IU/mL)	CV (%)
1	10	0.42	5.2	10	0.45	7.2
2	10	3.12	4.6	10	3.52	6.8
3	10	40.36	5.3	10	38.49	7.5

Table 4

Method comparison of CLIA-sIgE assay with Phadia FEIA mmunoCAP.

	CLIA-sIgE assay		
	Positive	Negative	Total
Phadia FEIA ImmunoCAP			
Positive	87	9	96
Negative	6	62	68
Total	93	71	164
χ^2	108.494 ($P < 0.05$)		
Kappa	0.813		

reveals a significant correlation between both assays ($r = 0.935$, $P < 0.001$). The linear regression equation is mapped with the FEIA values as the X-axis and the CLIA values as the Y-axis, the linear equation is $y = 1.0103x + 0.0408$, $r = 0.9729$ (x: IU/mL, y: IU/mL). The CLIA-sIgE assay shows good consistency with the Phadia ImmunoCAP FFIA system.

3.3.6. Interference

High levels of bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL) were introduced into the samples. All did not interfere with the test results. The bias was $<10.0\%$ (Table 5).

3.3.7. Cross-reactivity

The cross-reaction rates between allergic positive serum and recombinant Bet v1 are 73% (22/30), 78% (21/27), and 67% (20/30) for carrot, apple, and celery, respectively. The results are consistent with

other studies that the concordance rates of pollinosis complication in the OAS group were around 70%.

4. Discussion

Enzyme-linked immunosorbent assay (ELISA), FEIA, and CLIA are the most commonly used immunoassay formats for allergen-sIgE. ELISA has been widely used because of its convenience, high sensitivity, and specificity. In general, the LOD of ELISA for allergen sIgE is 0.35 IU/mL, which is set to be the critical point of allergen-sIgE. According to a consensus guidance document, the current recommendation for clinical laboratories is that sIgE levels must be reported as analytical measurements down to the cleared limit of quantitation of the assays, which is usually 0.1 IU/mL (Hamilton and Oppenheimer, 2015). The LOD and sensitivity of FEIA and CLIA are 2–8 fold better than that of ELISA. The Phadia ImmunoCAP FEIA is the current gold-standard method for the detection of allergen-sIgE. The CLIA method is automation and requires very small amounts of samples and reagents. It is a very popular *in vitro*

Table 5

Endogenous interference in serum for sIgE to rBet v1 detection.

Endogenous interference	Conc (mg/dL)	Control (IU/mL)	Without additives (IU/mL)	Bias (%)
Bilirubin	50	1.23	1.19	−3.3%
Hemoglobin	400	1.23	1.31	6.5%
Lipid	2000	1.23	1.28	4.1%

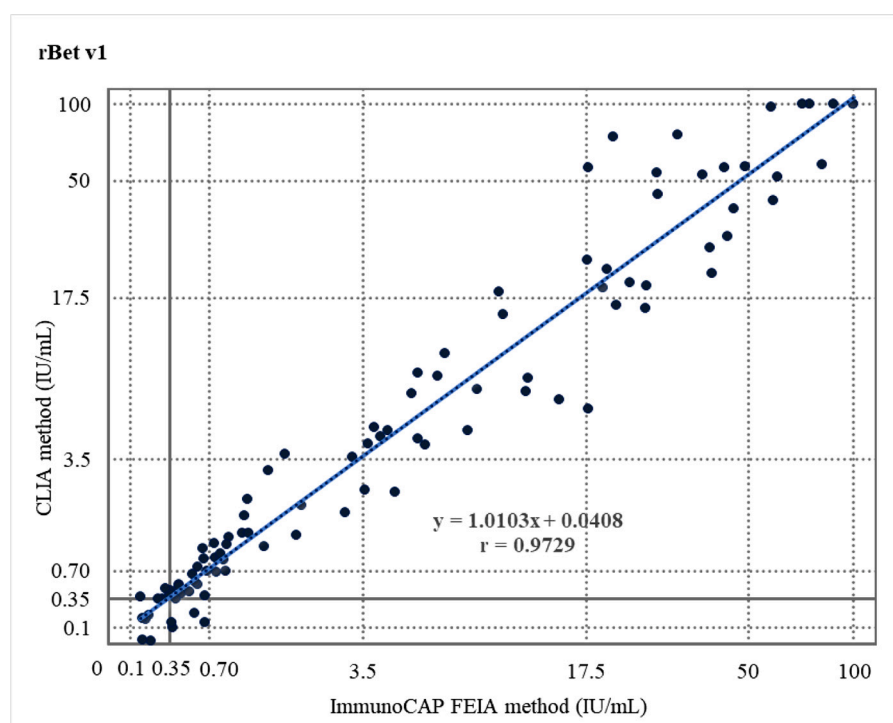


Fig. 7. Correlation between results measured by the proposed CLIA-sIgE assay and Phadia ImmunoCAP FEIA. Correlation coefficient of 0.935 ($P < 0.001$).

diagnosis due to the advantages of high-throughput, short time, and wide dynamic range. Especially, the CLIA method with high sensitivity, specificity, rapidity, and reproducibility has been widely proposed for the evaluation of biomarkers in human serum.

Molecular allergology is an analysis of component-specific, which helps to distinguish the specificity and cross-reactivity of patients with suspected multi-sensitization to various allergens. CRD is also particularly effective in patients with anaphylaxis, not only detecting the actual molecular components involved in the allergen, but also eliminating cross-reactive allergens and reducing adverse events (Melioli et al., 2011). Risk assessment is helpful to avoid trigger allergens and choose the appropriate strategies for allergen-specific immunotherapy.

OAS is a pollen-food allergy syndrome, which exhibits allergic reactions to fresh fruits or vegetables caused by pollen antigen sensitization (Carlson and Coop, 2019; Kashyap and Kashyap, 2015). Betula sensitized patients have mild symptoms after carrot, apple, celery, and other foods ingestion. The causative allergen is Bet v1, the main allergen component of birch pollen, which can provide sensitized patients with IgE specific for pan allergens and/or cross-reacting allergens. In this study, the cross-reaction rates of allergic positive serum of carrot, apple, and celery with recombinant Bet v1 were 73% (22/30), 78% (21/27), and 67% (20/30), respectively. The result is consistent with other studies that the coincidence rate of pollinosis complications in the OAS group is about 70% (Ciprandi et al., 2016; Katelaris, 2010).

The CLIA-sIgE assay agrees well with the reference method and is suitable for early birch pollen rBet v1 sIgE antibodies hypersensitivity or OAS. The CLIA-sIgE assay is also particularly powerful not only to detect the rBet v1 sIgE antibodies in the patients' serum, but also to help patients to avoid trigger allergens. Allergy diagnosis using recombinant allergen components matched CLIA method with high sensitivity and specificity is crucial for precision medicine.

5. Conclusion

In conclusion, a CLIA method for the detection of birch pollen allergic sIgE was established by using rBet v1 protein expressed by *E. coli*. The results of both CLIA and FEIA ImmunoCAP systems showed a substantial correlation for sIgE to rBet v1 detection. The good performance of the CLIA allows its application for the detection of sIgE to rBet v1 in serum samples, which can be a reliable immunoassay for laboratory birch pollen allergen diagnostic. The results are interchangeable between these two assays. The established CLIA is essential for clinical decision-making in allergy patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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