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# Evaluation of the clinical performance of multiple serum sIgE detection systems based on component-resolved diagnosis

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## Abstract

**Background:** Serum allergen-specific IgE (sIgE) detection is an important tool in the diagnosis of allergic diseases. However, the absence of international standards for sIgE detection systems raises questions about the comparability of different systems.

**Objective:** This study aims to evaluate three common allergen sIgE detection systems, with a primary focus on detecting dust mite allergens.

**Methods:** We recruited 85 children with rhinitis and 15 healthy control children. The subjects underwent testing with three different sIgE detection systems, including magnetic particle flow fluorescence, magnetic particle chemiluminescence, and protein chip, to detect sIgE levels to HDM extracts. In addition, skin prick testing (SPT) was conducted, and protein chip technology was performed to measure sIgE levels to component proteins.

**Results:** Our findings reveal strong consistency between SPT and the three *in vitro* detection systems, with consistency exceeding 71.76% for dust mite allergens. Moreover, there was excellent consistency and RAST class consistency among the three *in vitro* detection systems, with scores exceeding 94.12% and 89.00%, respectively. And for the 13 additional allergens crude extracts sIgE simultaneously detected by systems 1 and 2, the results showed that the consistency of both systems was above 87.00%, and the RAST class consistency was above 82.00%.

**Conclusion:** The three serum sIgE detection systems exhibited an approximate 80% concordance rate with SPT in identifying dust mite allergens. Furthermore, these systems demonstrated excellent consistency and RAST class consistency among themselves. These findings suggest that the three assays introduced in this study are interchangeable in allergen diagnosis.

**Key word:** Evaluate, sIgE, Clinical performance, Allergy, Serum sIgE detection systems

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## Introduction

In recent decades, the prevalence of allergic diseases has shown a continuous upward trend globally, affecting approximately 20% of the global population.[1] Allergic diseases encompass a common category of illnesses, with allergic rhinitis (AR), asthma, urticaria, among others, being the primary manifestations. The etiology of these diseases is complex and multifactorial, often involving various allergens such as dust mites, pollen, and animal fur.[2] To ensure accurate and prompt diagnosis and treatment of allergic diseases, identifying the allergens involved is a crucial aspect of clinical practice.

The diagnosis of allergic diseases is typically based on a patient's clinical history, combined with either *in vivo* skin prick tests (SPT) or *in vitro* allergen-specific IgE (sIgE) test results. However, current tests used for clinical allergy diagnosis have some limitations, such as high costs, lengthy procedures, and demanding skilled operators, making it difficult to be widely adopted in medium to low-resource healthcare facilities. Thus, the quest for alternative, cost-effective, and broadly applicable allergy diagnostic systems is imperative.

Different *in vivo* and *in vitro* allergen detection methods exhibit variations in detection efficacy, operational complexity, and cost. Hence, it is essential to compare and evaluate different detection methods so that clinicians can select allergen detection methods that are more accurate, convenient, and cost-effective. This study aims to compare three common serum sIgE detection Systems, including magnetic particle flow fluorescence, magnetic particle chemiluminescence, protein chip for detecting crude allergen sIgE levels. Additionally, protein chip was performed to measure sIgE levels for component proteins as a reference. By integrating clinical patient histories and SPT as complementary diagnostic tools, this study evaluates the efficacy of multiple systems for detecting IgE levels to mite allergens and explores the practical value of these systems in detecting allergen sIgE.

## Subjects and Methods

### *Study Subjects*

This study recruited 85 children with rhinitis and 15 healthy control children from the Department of Pediatrics and Physical Examination Department at the First Affiliated Hospital of Guangzhou Medical University between June 2022 and December 2022. The 85 children with rhinitis included in the study presented with at least two or more of the following symptoms: nasal congestion, clear rhinorrhea, nasal itching, and sneezing. Physical examination findings revealed pale nasal mucosa, edema, and clear rhinorrhea. The children ranged in age from 3 to 6 years old. Experienced clinical physicians assessed the severity of the four main rhinitis symptoms, including nasal itching, nasal congestion, continuous sneezing, and runny nose, using a rhinitis scoring scale.[3] Based on this assessment, the children were categorized into mild, moderate, and moderately severe groups.

Exclusion Criteria were as follows: 1) Acute upper respiratory tract infections, systemic or other allergic diseases, inflammatory or infectious diseases within the month preceding the study; 2) Coexistence of malignant tumors; 3) Severe immunological disorders, including autoimmune diseases and immunodeficiency diseases; 4) Cardiovascular diseases, liver or kidney dysfunction; 5) Use of antiallergic medications within 7 days before blood collection, systemic corticosteroid therapy, or immunotherapy in the month preceding blood collection. All healthy control subjects reported no discomfort, had no history of allergic diseases, and had no medical visits or medication use in the past month. Both the control children were matched with rhinitis children in terms of gender and age and were from the same geographical area.

This study and the use of participant biological samples were approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (Approval No. GYYY-2021-67). Written informed consent was obtained from the parents (for child participants) of all participants.

### *Skin Prick Test (SPT)*

In this study, standardized allergen extracts for *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) were used, which were manufactured by Beijing Macro-Union Pharmaceutical Co., Ltd. Histamine (10 mg/ml) and diluent were utilized as the positive and negative control, respectively. Allergen extracts and control solutions were put on the volar side of the forearm for SPT. After 15 minutes, the average value of the longest diameter and the length of the perpendicular line through its middle would be determined through the wheal reaction. When subtraction of the negative control, a positive skin reaction was defined as a wheal size ≥ 3 mm.

SIgE detection of allergen extractsIn this study, three different methods were employed for detecting serum sIgE antibodies against Der p and Der f of the participants. The methods used were magnetic particle flow fluorescence (MF1280, Dymind Co., Ltd., Shenzhen, China) (System 1), magnetic particle chemiluminescence (Sharay 4000, C-Luminary Biotechnology Co., Ltd., Sichuan, China) (System 2), and protein chip technology (DX-Blot 45II, Zheda Dixun Biological Gene Engineering Co., Ltd., Zhejiang, China) (System 3). Additionally, System 1 and System 2 were simultaneously employed to detect the sIgE levels against 13 other allergens, namely cat furry (*Felis domestics*, Fel d), dog furry (*Canis familiaris*, Can f), cockroach (*Blattella germanica*, Bla g), *Aspergillus fumigatus* (Asp f), *Alternaria alternata* (Alt a), common ragweed (*Ambrosia artemisiifolia*, Amb a), mugwort (*Artemisia vulgaris*, Art v), cow's milk (*Bos domesticus*, Bos d), codfish (*Gadus morhua*, Gad m), wheat (*Triticum aestivum*, Tri a), crab (*Cancer pagurus*, Can p), beef (*Bos spp*, Bos s), and lamb (*Ovis spp*, Ovi s).

The principle of the magnetic particle flow fluorescence detection system involves the specific binding of allergen sIgE antibodies in the sample with fluorescent microspheres coated with specific allergens. This forms an allergen-specific antibody-biotinylated mouse anti-human IgE monoclonal antibody immune complex. A reaction solution containing streptavidin-phycoerythrin (SAPE) binds to the biotin and streptavidin interaction on the fluorescent microspheres. The instrument automatically excites the fluorescent signal from the incubated reaction, and the concentration of sIgE antibodies is directly proportional to the fluorescence intensity. The concentration of sIgE antibodies in the sample is calculated based on the fluorescence signal using a calibration curve.

The magnetic particle chemiluminescence detection system operates based on an indirect method of magnetic particle chemiluminescence immunoassay. In this method, the test sample is mixed with magnetic microspheres and biotin-labeled allergens, followed by a 10-minute incubation. After washing, alkaline phosphatase (ALP)-labeled anti-human IgE secondary antibodies are added and allowed to react for another 10 minutes. This results in the formation of a solid-phase antigen-antibody-enzyme-labeled secondary antibody complex. Unbound enzyme-labeled antibodies and other substances are removed through washing steps. Subsequently, a chemiluminescent substrate, 3-[2-spiroadamantane]-4-methoxy-4-[3-phosphoryloxy]-phenyl-1,2-dioxetane (AMPPD), is introduced. Under the catalytic action of alkaline phosphatase (ALP), this substrate emits lights, and the and the intensity of the light signal is directly proportional to the concentration of the corresponding antigen-specific IgE in the sample.

The principle of the protein chip detection system involves immobilizing antigens on a nitrocellulose membrane. When serum containing allergen-specific sIgE antibodies is added to the detection plate, these sIgE antibodies bind to the immobilized antigens on the membrane, forming immune complexes of sIgE antibodies and antigens that are fixed on the membrane. Any substances that did not bind to the membrane are removed through a washing step. Subsequently, a biotin-conjugated anti-human IgE antibody complex is added to the detection plate, resulting in the formation of a complex comprising antigen-sIgE antibody-anti-human IgE antibody-biotin. After washing to remove unbound substances, in the third reaction step, an ALP-streptavidin conjugate is added to the detection plate. This leads to the formation of a new immune complex: antigen-sIgE antibody-anti-human IgE antibody-biotin-streptavidin-ALP complex. Following the removal of unbound substances through washing, a substrate solution BCIP/NBT is added for a colorimetric reaction. The intensity of color in the lines/dots represents the concentration of sIgE antibodies present in the serum.

In theory, these three systems are capable of detecting all types of allergens. However, they must obtain a registration certificate from the National Medical Products Administration before they can be clinically used. Generally speaking, for common inhalant and food allergens, all three systems have obtained the necessary licenses and are widely used in clinical practice in China.

### *Total IgE Detection*

Serum total IgE (tIgE) levels were measured using two methods, System 1 and System 2.

### *Dust Mite Allergen Components sIgE Detection*

Serum sIgE levels for seven Der p allergen components (Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 21, Der p 23) and two Der f components (Der f 1, Der f 2) were measured using the protein chip system (System 3).

### *Definition*

The sIgE levels of allergen crude extract and components proteins sIgE are expressed in kilo International Units per liter ($kU_A/L$), with a detection range of 0.35-100 $kU_A/L$. SIgE levels less than 0.35 $kU_A/L$ are defined as negative. Based on RAST classification, levels of sIgE were quantitatively categorized into six classes: Class 0, < 0.35 $kU_A/L$; Class 1, 0.35–0.70 $kU_A/L$; Class 2: 0.70–3.50 $kU_A/L$; Class 3: 3.50–17.50 $kU_A/L$; Class 4: 17.50–50.00 $kU_A/L$; Class 5: 50.00–100.00 $kU_A/L$ and Class 6: ≥ 100.00 $kU_A/L$. ±1 class consistency refers to the proportion of cases where the difference in classes between two allergens or two systems does not exceed 1 class. TIgE levels are expressed in kilo International Units per liter (kU/L), with a detection range of 2-5000 kU/L.

### *Statistical analysis*

Data organization was conducted using Excel 2019 (Microsoft® Excel® 2019), and data analysis was performed using GraphPad Prism 8.0.2 (© 1992-2019 GraphPad Software, Inc.) and R Studio 2022.07.2 Build 576 (© 2009-2022 RStudio, PBC). Adobe Illustrator CC 2015.0.0 (Adobe Inc.) was employed for image retouching and integration. Binary data were presented as frequencies (percentages), and the chi-square test ($\chi^2$) or Fisher's exact probability test was employed to compare the distribution differences among groups. Parametric quantitative data were expressed as mean ± standard deviation, while nonparametric quantitative data were represented as median (interquartile range). Binary indicators, including consistency rate, positive agreement rate, and negative agreement rate, were utilized to assess the concordance between different detection systems. And Kendall's tau test was employed to analyze the correlations between different systems or different allergen items. $P < 0.05$ was considered statistically significant.

## Results

### *Characteristics of the study subjects*

This study included a total of 85 children with AR and 15 healthy control children. Overall, the proportion of male children was slightly higher than that of female children (56.0% vs. 44.0%), with a median age of 5.00 (4.00, 5.00) years. There were no statistically significant differences in age or gender between the children in the rhinitis and control groups. Among the children in the AR group, the proportions of mild, moderate, and moderately severe symptoms were 42.0%, 34.0%, and 9.0%, respectively. SPT results indicated that 61.2% of patients were positive for house dust mites, and 48.2% of children were positive for storage dust mites. Both System 1 and System 2 showed significantly lower tIgE levels in the healthy control group compared to the AR group (18.30 [6.90, 41.75] vs. 130.82 [28.98, 317.48], and 19.53 [12.48, 80.10] vs. 216.23 [54.73, 522.85], respectively; both $P < 0.001$) (**Table 1**).

**Table 1. Demographic characteristics of the participants.**

| Variable | Overall | HC | Rhinitis | *P*-value |
|---|---|---|---|---|
| Sample size | 100 | 15 | 85 | |
| Sex (%) | | | | |
| Male | 56 (56.0) | 8 (53.3) | 48 (56.5) | 1.000 |
| Female | 44 (44.0) | 7 (46.7) | 37 (43.5) | |
| Age (median [IQR]) | 5.00 [4.00, 5.50] | 4.00 [4.00, 5.00] | 5.00 [4.00, 5.50] | 0.071 |
| severity (%) | | | | |
| Normal | 15 (15.0) | 15 (100.0) | 0 (0.0) | < 0.001 |
| Mild | 42 (42.0) | 0 (0.0) | 42 (49.4) | |
| Moderate | 34 (34.0) | 0 (0.0) | 34 (40.0) | |
| Moderately severe | 9 (9.0) | 0 (0.0) | 9 (10.6) | |
| SPT Der p (%) | | | | |
| Negative | 48 (48.0) | 15 (100.0) | 33 (38.8) | < 0.001 |
| Positive | 52 (52.0) | 0 (0.0) | 52 (61.2) | |
| SPT Der f (%) | | | | |
| Negative | 58 (58.0) | 15 (100.0) | 44 (51.8) | 0.003 |
| Positive | 42 (42.0) | 0 (0.0) | 41 (48.2) | |
| tIgE (median [IQR]) | | | | |
| System 1 | 101.45 [22.35, 290.84] | 18.30 [6.90, 41.75] | 130.82 [28.98, 317.48] | < 0.001 |
| System 2 | 165.05 [37.77, 408.06] | 19.53 [12.48, 80.10] | 216.23 [54.73, 522.85] | < 0.001 |

**Note:** HC-healthy control; SPT-skin prick test. We compared proportions between groups using the chi-squared test ($\chi^2$) and performed intergroup comparisons for non-parametric quantitative data using the Kruskal-Wallis H test. Positivity for dust mites allergen was determined by skin prick testing.

### *Comparison of Consistency Among Multiple Allergen Detection Systems*

For Der p and Der f, both SPT and three serum sIgE detection systems were conducted. The consistency and RAST class consistency among them were evaluated (**Table 2**). Regarding Der p, all three *in vitro* sIgE detection systems exhibited excellent consistency among their results, exceeding 94.12%, and their consistency with SPT ranged above 87.06%. For Der f, the consistency among the results from the three *in vitro* sIgE detection systems exceeded 94.12%, while their consistency with SPT ranged between 71.76% and 78.00%. Both dust mite allergens demonstrated strong positive and negative consistency among different systems. However, the positive consistency of *in vitro* detection systems 1 and 2 with SPT was only 63.79% and 65.00%, respectively, and the negative consistency of system 3 with SPT was 47.83%. Further analysis was performed to assess the RAST class consistency among the systems. The RAST class consistency among the results of Der p and Der f sIgE detected by the three systems ranged from 57.65% to 68.00%, while the ±1 class consistency was consistently above 89.00%. Quantitative correlation analysis of the sige of Der p and Der f detected by the three systems shows that the correlation of Der p between the different systems exceeds 0.900 (and there is a considerable correlation between Der f detected by the different systems). Additionally, for the 13 additional allergen crude extracts sIgE simultaneously detected by systems 1 and 2, the results showed that the consistency of both systems was above 87.00%, and the RAST class consistency was above 82.00%.

**Table 2. Comparison of consistency between skin prick testing and three serum sIgE detection systems.**

| Characteristics | Der p | | | Der f | | |
|---|---|---|---|---|---|---|
| | System 1 | System 2 | System 3 | System 1 | System 2 | System 3 |
| Consistency | | | | | | |
| System 2 | 98.00% | - | - | 98.00% | - | - |
| System 3 | 94.12% | 96.47% | - | 94.12% | 96.47% | - |
| SPT | 90.00% | 92.00% | 87.06% | 77.00% | 78.00% | 71.76% |
| Positive consistency | | | | | | |
| System 2 | 100% | - | - | 96.67% | - | - |
| System 3 | 100.00% | 100.00% | - | 100.00% | 100.00% | - |
| SPT | 86.21% | 86.67% | 82.54% | 63.79% | 65.00% | 100.00% |
| Negative consistency | | | | | | |
| System 2 | 95.24% | - | - | 95.24% | - | - |
| System 3 | 81.48% | 88.00% | - | 81.48% | 88.00% | - |
| SPT | 95.24% | 100.00% | 100.00% | 92.86% | 97.50% | 47.83% |
| Class consistency | | | | | | |
| System 2 | 68.00% | - | - | 56.00% | - | - |
| System 3 | 63.53% | 57.65% | - | 60.00% | 30.59% | - |
| ±1 Class consistency | | | | | | |
| System 2 | 89.00% | - | - | 86.00% | - | - |
| System 3 | 90.59% | 95.29% | - | 90.59% | 64.71% | - |
| Quantitative correlation* | | | | | | |
| System 2 | 0.927 | - | - | 0.931 | - | - |
| System 3 | 0.918 | .950 | - | 0.934 | 0.931 | - |

**Note:** System 1- magnetic particle flow fluorescence detection system; System 2- magnetic particle chemiluminescence detection system; System 3- protein chip detection system. SPT-skin prick test. Der p-*Dermatophagoides pteronyssinus*. Der f-*Dermatophagoides farinae*. *Quantitative correlation analysis was performed using spearman`rho.

### *Analysis of Consistency Between Dust Mite Crude Extracts and Dust Mite Component Proteins*

System 3 was also utilized to measure the sIgE levels of components (Der p 1, Der f 1, Der p 2, Der f 2, Der p 5, Der p 7, Der p 10, Der p 21, Der p 23) in 85 rhinitis patients. Among these 85 rhinitis patients, the positive rates for Der p 1, Der f 1, Der p 2, Der f 2, Der p 7, Der p 21, and Der p 23 ranged from 37.65% to 64.71%, while the positive rates for Der p 5 and Der p 10 were comparatively lower (**Figure 1**). Notably, among samples that were simultaneously positive for Der p and Der f, Der p 1 and Der f 1 exhibited high positivity rates of 85.71% and 87.30%, followed by Der p 2 and Der f 2 (73.02% and 79.37%). Der p 21 (69.84%), Der p 7 (57.14%), and Der p 23 (49.21%) also showed relatively high positive rates.

To validate comparability of various methods in allergen detection, comparisons of consistency between the three *in vitro* systems' detection of Der p and Der f with various components were conducted. As shown in **Figure 2**, regardless of whether the consistency is high or low, the three systems demonstrate similar levels of agreement with the individual components. Overall, systems 1 and 2 demonstrated substantial consistency with various dust mite components, slightly outperforming system 3. Furthermore, we performed Kendall's tau correlation analysis of the semi-quantitative correlation between the allergen crude extracts and components detected by the three systems (**Figure 3A**), as well as ±1 class consistency analysis (**Figure 3B**). Kendall's tau correlation analysis and ±1 class consistency analysis revealed correlation coefficients ranging from 0.85 to 0.93 between the dust mite allergens Der p and Der f detected by the three *in vitro* systems, with ±1 class consistency exceeding 96%. Similarly, the correlation coefficients between different systems for the same dust mite components ranged from 0.79 to 0.89, with ±1 class consistency ranging from 84.71% to 100%.

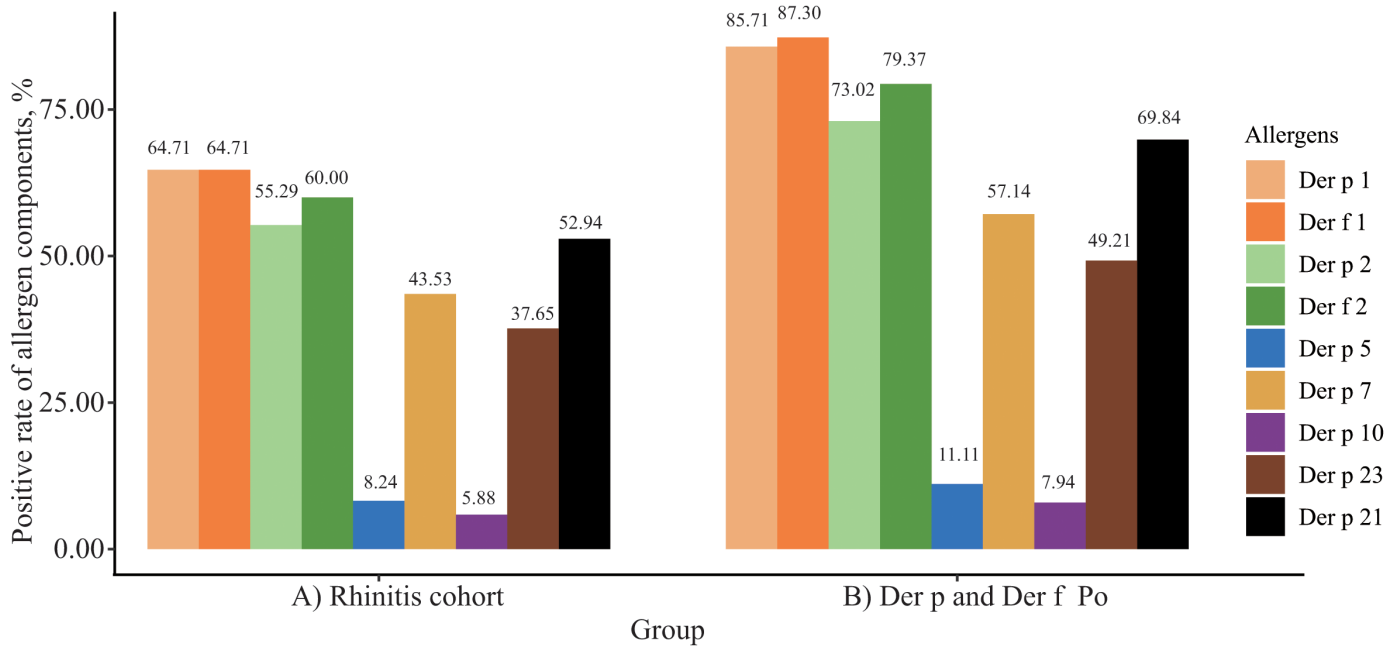


**Figure 1.** Sensitization rates to two dust mite components among the rhinitis cohort. A) All rhinitis patient cohort, and B) Rhinitis patients positive for both Der p and Der f.

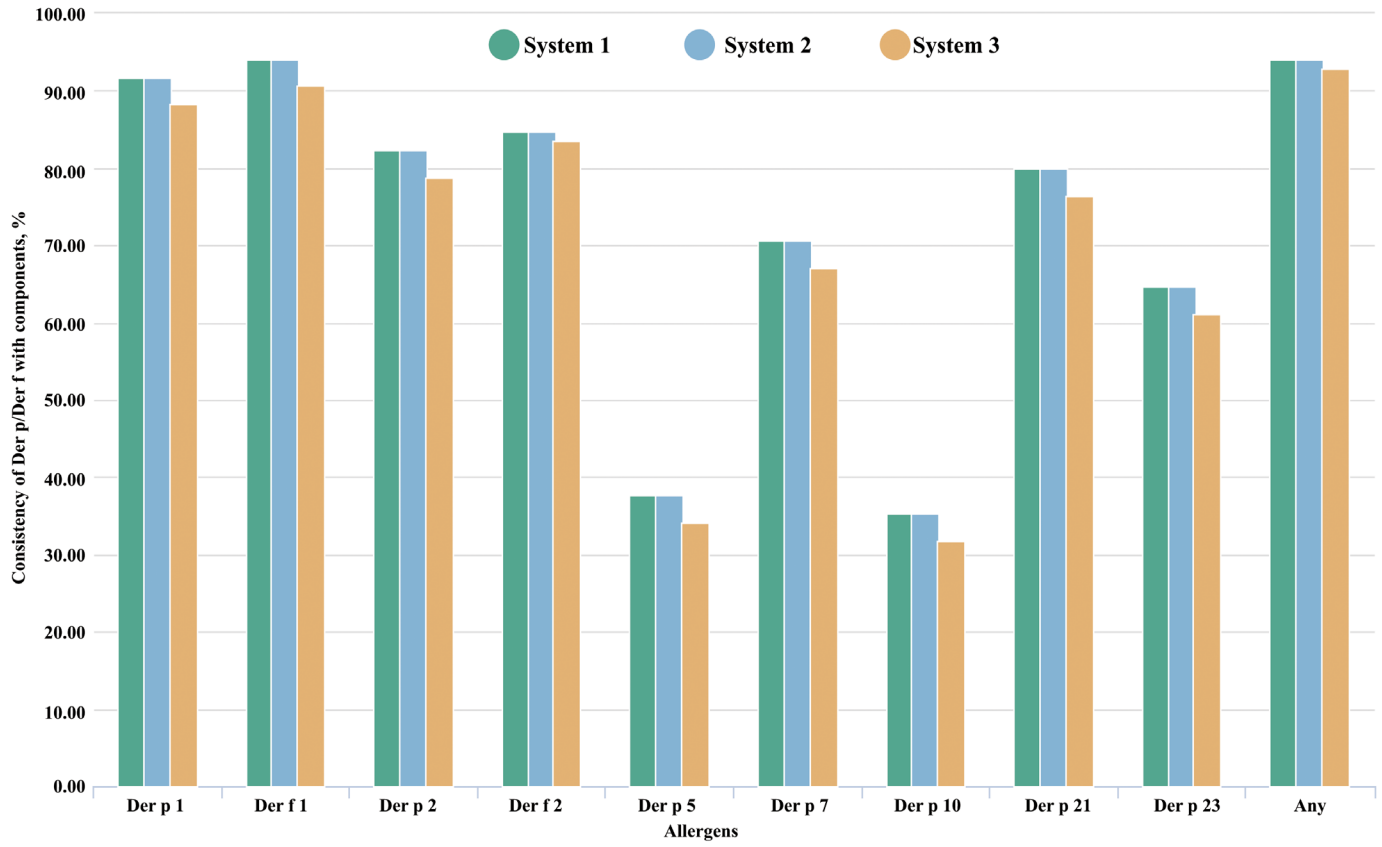


**Figure 2.** Consistency among Der p crude extract and components detected by three in vitro systems. "Any" indicates that positivity for any component is considered overall positive, while negativity for all components is regarded as overall negative.

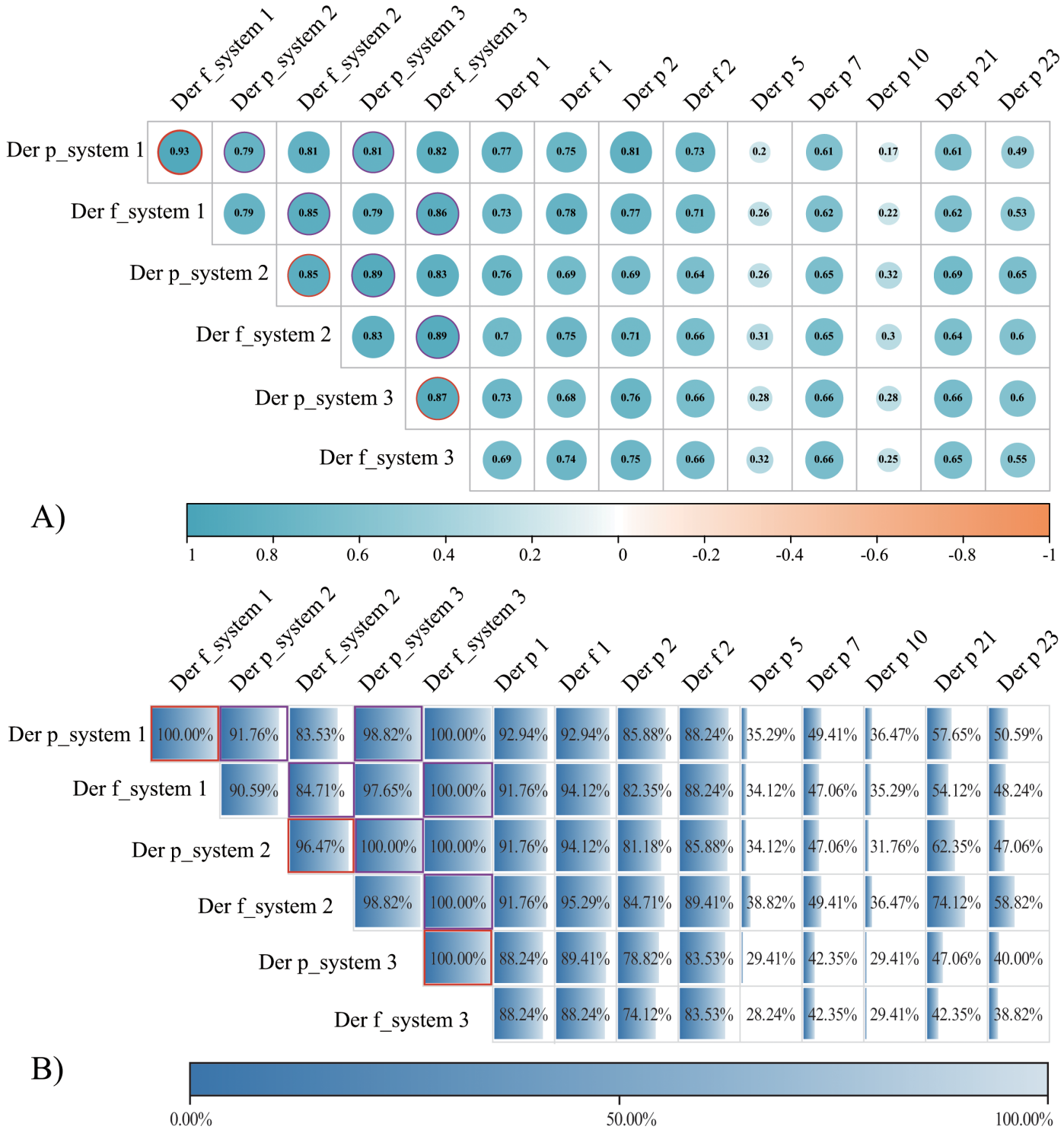


**Figure 3.** Analysis of correlation and ±1 class consistency between Der p/Der f and dust mite components detected by three in vitro systems: A) Correlation analysis using the Kendall' tau method, and B) ±1 class consistency, which refers to the proportion of cases where the difference in classes between two allergens or two systems does not exceed 1 class.

**Table 3. Cases of inconsistent results for the Der p and Der f among multiple systems.**

| Patients | Diagnostic | Severity | Der p | | | | | Der f | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SPT | System 1, $KU_A/L$ | System 2 $KU_A/L$ | System 3 $KU_A/L$ | Der p 1/2/7/10/21/23 | SPT | System 1, $KU_A/L$ | System 2 $KU_A/L$ | System 3 $KU_A/L$ | Der f 1 $KU_A/L$ | Der f 2 $KU_A/L$ |
| No. 1 | rhinitis | mild | Ne | 0.20 | 0.81 | 9.6 | Both Ne | Po | 0.17 | 0.65 | 11.01 | 0.07 | 0.02 |
| No. 2 | rhinitis | mild | Po | 0.12 | 1.02 | 7.8 | Both Ne | Po | 0.14 | 0.78 | 7.99 | 0.08 | 0.46 |
| No. 3 | rhinitis | mild | Ne | 0.10 | 0.29 | 1.38 | Both Ne | Ne | 0.10 | 0.22 | 0.46 | 0.07 | 0.04 |
| No. 4 | rhinitis | mild | Ne | 0.15 | 0.12 | 1.2 | Both Ne | Ne | 0.31 | 0.1 | 0.98 | 0.06 | 0.73 |
| No. 5 | rhinitis | moderate | Ne | 0.10 | 0.10 | 0.45 | Both Ne | Ne | 0.10 | 0.10 | 0.41 | 0.05 | 0.07 |

**Note:** System 1- magnetic particle flow fluorescence detection system; System 2- magnetic particle chemiluminescence system; System 3- protein chip detection system. Der p-*Dermatophagoides pteronyssinus*. SPT-skin prick test. Der f-*Dermatophagoides farinae*. Red font indicates negative, and black font indicates positive.

Furthermore, both correlation and class consistency analyses indicated a strong correlation (0.66-0.81) and ±1 class consistency (74.12%-92.94%) between the crude extracts and Der p/f 1/2 components, followed by Der p 7, Der p 21, and Der p 23. From a qualitative and semi-quantitative perspective, we introduced allergenic components to analyze the three systems found to be highly interchangeable.

### *Case Analysis of Inconsistent Detection Results Among Systems*

Further analysis revealed that five rhinitis patients exhibited contradictory detection results across multiple systems. As shown in **Table 3**, for instance, Patient No. 1's Der p results were negative in SPT, System 1, and all Der p components but positive in System 2 and 3. Patient No. 2's Der p results were negative in System 1 and all Der p components but positive in SPT, System 2, and System 3. Patients No. 3, No. 4, and No. 5 displayed negative Der p results in SPT, System 1, System 2, and all Der p components but positive results only in System 3. Conversely, the same five rhinitis patients exhibited inconsistent results for Der f (**Table 3**). For example, Patient No. 1's Der f results were negative in System 1 and Der f 1, Der f 2 components but positive in SPT, System 2, and 3. Patient No. 2's Der f results were negative only in System 1 but positive in SPT, System 2, System 3, and Der f 2 components. Patients No. 3 and No. 5 displayed negative Der f results in SPT, System 1, System 2, and Der f 1, Der f 2 components but positive results only in System 3. Patient No. 4's Der f results were negative in SPT, System 1, and 2 but positive in System 3 and Der f 2 component.

## Discussion

In recent years, serum allergen- sIgE detection has become an important tool in the diagnosis of allergic diseases in clinical practice due to its advantages, including minimal interference, high accuracy, and effective relief of patient discomfort.[4] However, there is a lack of international standards for allergen sIgE detection systems, particularly in terms of reagent materials, and it remains unclear whether sIgE results provided by different manufacturers using varying detection methods and materials are comparable.[5] In this study, we evaluated three different serum sIgE detection systems for dust mite. Additionally, in contrast to previous studies, we incorporated detection systems for dust mite components proteins.

For two mite allergens, three different in vitro testing systems demonstrated good consistency with SPT, consistent with previous research findings.[6] Furthermore, these systems showed consistency of over 94.12%, with good consistency observed in both positive and negative sample comparisons. The ±1 class indicates that the difference in classes between two systems' detected results does not exceed one class. This metric was introduced to assess the class of consistency in test results among different systems, and the results showed that all systems also exhibited good ±1 class consistency (close to 90%) at a semi-quantitative level. Due to cross-reactivity between house dust mites and storage dust mites, they may exhibit similar patterns in both methods,[7] so we also detected additional 13 allergens with systems 1 and 2, all of which showed good consistency and RAST class consistency.

The first and second groups of dust mite allergens (Der p 1 and Der f 1, Der p 2 and Der f 2) are considered the most clinically relevant HDM allergens.[8] This study found that Der p 1, Der p 2, Der f 1, and Der f 2 had the highest sensitization rates and are the main sensitizing components of dust mites, similar to conclusions from related studies in Guangdong province[9,10] and Wuhan region,[11] China. Our study indicates that Der p 5 and Der p 10 functions as a minor allergen in our studied population, with a relatively low sensitization rate. This finding aligns consistently with previous research outcomes.[12] It is noteworthy that among patients who were simultaneously positive for both two dust mites in this study, the positive rates for Der p 21, Der p 23, and Der p 7 were 69.84%, 49.21%, and 54.00%, respectively, significantly higher than the results reported by Wang et al. in northern China.[13] Some studies have shown that the positive rate of Der p 23 is positively correlated with symptoms.[14] The significantly higher sensitization rate in the southern region may be due to our calculation of sensitization in patients who were positive for both two dust mites, and 50% of the patients had moderate to severe rhinitis. Additionally, this suggests that different regions may have different sensitization rates for dust mite components, and there may be regional differences in sensitization rates for Der p 21, 23, and Der p 10.

In this study, regardless of whether the consistency is high or low, the three systems demonstrate similar levels of agreement with the individual components with System 1 and System 2 in perfect agreement, slightly outperforming System 3, suggesting that the three system systems are interchangeable. The three systems showed a high correlation between Der p and Der f, with a relatively consistent ±1 class. Moreover, we found that the main components of dust mite allergens were superior to other components in terms of ±1 class consistency and correlation between crude extracts and components.

In summary, the three different in vitro systems generally performed well in the detection of dust mite allergens, supporting the comparability of various methods in allergen detection. However, we also noted occasional discrepancies in results among different systems in a very small number of patients, which may be influenced by multiple factors. These discrepancies could be attributed to differences in the methodologies, underlying principles, variations in the microenvironment of antigen-antibody reactions due to antigen coating on distinct carriers, and significant disparities in signal amplification and sensitivity among different allergen detection systems.[15] To arrive at a comprehensive assessment of whether a patient is genuinely sensitized, we integrated clinical symptoms, SPT, results from the three in vitro systems, and components findings. For example, in the case of patients with rhinitis numbered 2, all three methods (SPT, system 2, and system 3) detected positivity for both Der p and Der f, suggesting a higher likelihood of genuine sensitization. Conversely, for patients numbered 3 to 5, only system 3 yielded positive results, indicating a higher likelihood of false positives. On the other hand, when discrepancies between crude extract and component-specific results occurred, it does not imply the inaccuracy of a particular result; rather, it could be due to the patient's sensitization to components that were not part of the detection panel.[16] It is crucial to emphasize that sIgE detection typically serves as a supplementary diagnostic tool, and in cases where results are contentious, clinicians should consider the patient's clinical symptoms, medical history, and other relevant test outcomes to make a more accurate assessment. Therefore, our research findings should be regarded as a reference rather than a standalone decision-making basis.

However, it's also imperative to acknowledge certain limitations in our study. Firstly, our comparative analysis focused solely on dust mite allergens and did not encompass a systematic comparison of other allergens. While dust mite allergies are prevalent in clinical practice, the distinct immunological characteristics of other allergen sources may influence the comparison of different detection methods. Consequently, the cautious application of our study results to other allergens is warranted. Secondly, the overall sample size was relatively modest, which could potentially impact the stability and reliability of the results in certain scenarios. Future research should explore comparisons with other allergens, expand the sample size, and undertake more in-depth analyses that consider the clinical context to better comprehend the applicability of these in vitro systems across various allergens and clinical settings.

## Conclusions

In conclusion, the three in vitro testing systems exhibited an approximate 80% concordance rate with SPT in identifying dust mite allergens. Furthermore, these three in vitro testing systems demonstrated excellent consistency and RAST class consistency among themselves. These findings suggest that the three assays introduced in this study are interchangeable in allergen diagnosis.

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## Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

## Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYYY-2021-67). All subjects (children) through their parents, provided their written informed consent.

## Author Contributions

- Baoqing Sun, Huimin Huang and Zhifeng Huang conceived and designed the study, and obtained approval.
- Ziyu Yi, Tong Chen, Hui Gan, Zhiwei Lin and Mingshan Xue performed the detection of tIgE and sIgE for all samples.
- Zhifeng Huang, Aoli Li and Ziyu Yi analyzed the data.
- Zhifeng Huang and Aoli Li drafted the manuscript in close collaboration with all co-authors.
- All authors have read and approved the final version of the manuscript.

## Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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