# Efficacy of Allergen-Arrayed Chips for Detecting Allergen-Specific Immunoglobulin E in Allergic Patients

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***Objectives.*** When routine medical management of allergies is inadequate, diagnostic testing by the UniCAP® system or the skin prick test is often recommended to characterize the nature of the symptoms and to provide more specific therapies directed toward the identified sensitivities. The allergen-arrayed chip, which is based on a microarray technique, is a new method for detecting allergen-specific immunoglobulin (Ig) E. The aim of this study was to evaluate the efficacy of allergen-arrayed chips.

***Methods.*** A robotic microarrayer made the allergen-arrayed chips by spotting allergen extracts on poly-L-lysine microscopic glass slides. We enrolled 30 patients (19 men and 11 women) with asthma, allergic rhinitis or atopic dermatitis in this study. The serum specimens of allergic patients were tested by allergen-arrayed chips and the UniCAP® system for *Dermatophagoides pteronyssinus* (Dp), cockroach, egg white, milk, and shrimp allergens. The results were compared to assess the correlation, sensitivity and specificity.

***Results.*** The correlation coefficients between allergen-arrayed chips and the UniCAP® system for Dp, cockroach, egg white, milk, and shrimp were 0.92, 0.60, 0.63, 0.51, and 0.57, respectively. The allergen-arrayed chips showed high sensitivity (95%, 100%, 100%, 100%, and 83%, respectively) and specificity (100%, 83%, 86%, 97%, 83%, respectively) compared with the UniCAP® system.

***Conclusions.*** Clinically, the information provided by allergen-arrayed chips seems to be similar to that provided by the UniCAP® system. Moreover, the allergen-arrayed chips allow for the determination and monitoring of large numbers of disease-eliciting allergens by single tests and with minute amounts of sera. **( Mid Taiwan J Med 2004;9:96-102 )**

**Key words**

allergen, allergen-arrayed chips, allergen-specific IgE, *Dermatophagoides pteronyssinus*

## INTRODUCTION

Allergic diseases, such as asthma, allergic rhinitis and atopic dermatitis, affect more than 20% of the world population. The prevalence of these allergic diseases has been increasing significantly worldwide in recent decades [1-5]. Although most of these diseases are not life-threatening, they affect health and quality of life [6,7]. Avoidance of recognized allergens should

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minimize the impact of these allergic diseases, so identification of disease-eliciting allergens is important. Over the years, a number of methods have been developed to assess allergen-specific immunoglobulin E (IgE) in order to diagnose allergic diseases.

Causative allergens can be determined by appropriate provocation tests, skin tests and various immunoassays. However, provocation tests can be dangerous and unpleasant for the patient. Skin tests pose lower risks to patients than provocation, but anaphylaxis is still a possible side effect. Dermatographism or severe skin conditions, such as eczema or urticaria, and certain pharmacologic agents can interfere with skin responsiveness. In addition, these methods test only a limited number of allergens at a time. *In vitro* immunoassays, such as the radioallerosorbent (RAST) test first described in 1967 [8], was developed to measure specific IgE antibodies. For safety reasons, the radioactive label used in the earlier RAST assay has been replaced with enzyme labels that generate color [9,10] or fluorescence [11]. More recently, the Pharmacia CAP system, an updated version of the RAST assay, was introduced [12]. *In vitro* assays to detect allergen-specific IgE have been used along with or in place of skin-prick testing for more than 30 years.

Protein microarray was developed based on well-established DNA microarray technology, and a variety of applications have been demonstrated in several reports [13-16]. Microarrays of defined antigens have been used to detect allergen-specific IgE from human serum [17-19]. The microarray-based measurement of allergen-specific IgE allows for the determination and monitoring of large numbers of disease-eliciting allergens by single tests that require minute amounts of sera.

The aim of the present study was to detect multiple allergen-specific IgE antibodies to the house dust mite (*Dermatophagoides pteronyssinus*), cockroach, egg white, milk and shrimp by allergen-arrayed chips, and to compare the results with those obtained from the UniCAP® system.

## MATERIALS AND METHODS

### Patient Sera

A total of 30 patients referred to the allergy clinic at the China Medical University Hospital for evaluation of allergic symptoms were enrolled in this study. Informed parental and/or patient consent was obtained, and the study was approved by the Institutional Review Board of the China Medical University Hospital. The consensual consecutive patients included 19 men and 11 women (age range, 2 to 77 years; mean age, 21.7 years). Fourteen patients had allergic rhinitis, nine had asthma, five had both allergic rhinitis and asthma, and two had atopic dermatitis, allergic rhinitis and asthma. Venous blood was sampled from all patients and the patients' sera were stored at –20 °C until analysis. The blood levels of specific IgE to *Dermatophagoides pteronyssinus* (Dp), cockroach, egg white, milk and shrimp were measured by the UniCAP® method [20] and allergen-arrayed chips.

### Preparation of Allergen Proteins

Allergen powder, which included Dp, shrimp, egg white, milk and cockroach (Allergon® Inc, Angelholm, Sweden), was spotted triplicately on each chip. Human IgE and actin were also spotted on each chip as positive and negative controls respectively. The powder was suspended in Tween-20 and phosphate-buffered saline (PBS) at 1:1 w/v, mixed for several minutes, and then shaken for 30 minutes at 4 °C. The insoluble residues were removed by centrifugation at 12,000 rpm. The suspension was then stored at –20 °C before use.

### Preparation of Allergen-Arrayed Chips

Allergen extracts were dissolved in PBS containing glycerol and dimethylsulfoxide, and then transferred to 96-well plates for spotting. The prepared protein solutions were spotted on poly-L-lysine glass slides by Array-07, a robotic microarrayer (Wittech Co. Ltd, Taiwan, ROC), in a programmed order. The allergen-arrayed chips were then baked for 2 hours in a hot oven and stored in a dry box.

### Reaction

The allergen-arrayed chips were immersed

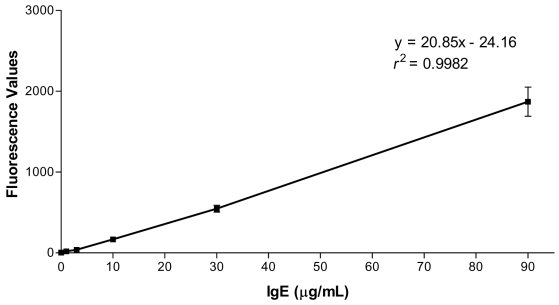


Fig. 1. The calibration curve of the allergen-arrayed chip. The standard curve was drawn from serially diluted semi-purified human IgE (90, 30, 10, 3, 1 μg/mL) and the average fluorescence intensities generated by the allergen-arrayed chip. Actin was chosen as the negative control.

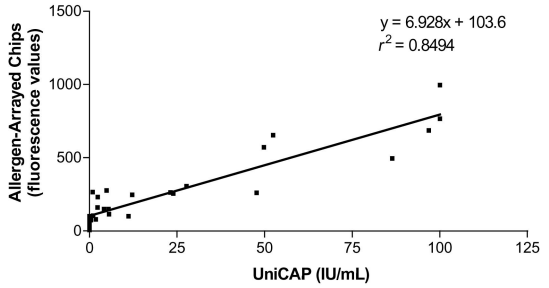


Fig. 2. Correlation between the quantity of serum Dp-specific IgE measured by UniCAP® and fluorescence intensities detected by the allergen-arrayed chip. Linear correlation is shown as y = 6.928x + 103.6, $r^2$ = 0.8494. (n = 30)

for 120 minutes in 1.5% bovine serum albumin solution containing 1.5% non-fat milk at room temperature in PBS to block the unreacted sites. After washing twice with PBS, the chips were immersed for 2 hours in 80 μL of the patient's serum. The allergen chips were then washed twice with PBS, and incubated with anti-human IgE-biotin for 2 hours at room temperature. The chips were then washed twice with PBS and incubated with streptavidin-cyanine (Cy)5 for 2 hours at room temperature in a dark room. Next, they were washed twice with PBS and then washed twice with double deionized water. Finally, the chips were dried by nitrogen gas.

### Fluorescence Detection

The reacted allergen-arrayed chips were scanned by a GenePix 4000B scanner (Axon Instrument Inc, California, USA) to detect fluorescence intensities generated by Cy5-conjugated streptavidin. The ScanArray was set at 635-nm excitation and 680-nm emission to visualize the Cy5-tagged proteins. Slide images were analyzed by GenePix 4.0 software (Axon Instrument Inc, California, USA).

### Subjects and Allergen-Arrayed Chip Set Order

For calibration purposes, pooled human IgE solutions were used to determine the best condition for generating fluorescence intensities. The results of the allergen-arrayed chip system were classified into 7 categories ranging from 0 to 6 based on fluorescence intensities. The classes of allergen chips were scored as follows: 0 to 49 (class 0); 50 to 99 (class 1); 100 to 199 (class 2); 200 to 299 (class 3); 300 to 499 (class 4); 500 to 799 (class 5); and above 800 (class 6). Fluorescence values of more than 100 (class ≥ 2) were considered positive. Depending on the classified score, 30 sera from allergic patients were tested and the results were analyzed to evaluate the reliability of the allergen-arrayed chip.

### Statistical Methods

The data were analyzed by the SPSS version 10.0. The Pearson's correlation coefficients and linear regression analysis were performed in this study. The sensitivity and specificity were calculated by standard methods.

## RESULTS

### Significance of Calibrator

We tested the correlation of fluorescent values with different IgE levels by the commercial semi-purified human IgE (Chemicon Inc, California, USA) (Fig. 1). Fluorescent intensity of spots increased linearly with the rise in IgE concentration. On the calibration curve, some non-specific intensity of the negative control was found, but it was always lower than that of the positive control.

### Correlation of Allergen-Arrayed Chips with UniCAP®

The serum of each patient was tested by both allergen-arrayed chips and the UniCAP® system. The correlations between allergen-arrayed chips and UniCAP® are listed in Table 1. The Pearson's correlation coefficients for Dp,

**Table 1. Correlation between allergen-arrayed chips and UniCAP® system**

| Allergen | Numbers | Pearson's correlation coefficient |
|---|---|---|
| Dp | 30 | 0.92** |
| Shrimp | 29 | 0.57** |
| Egg white | 30 | 0.63** |
| Milk | 30 | 0.51* |
| Cockroach | 30 | 0.60** |
| Combined | 149 | 0.85** |

Dp = *Dermatophagoides pteronyssinus*. *$p < 0.01$, **$p < 0.001$.

**Table 2. Comparison of allergen-arrayed chips with UniCAP® system**

| Allergen | No. of blood samples | Positive allergen-arrayed chips | | Negative allergen-arrayed chips | | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|
| | | Positive UniCAP® | Negative UniCAP® | Positive UniCAP® | Negative UniCAP® | | |
| Dp | 30 | 20 | 0 | 1 | 9 | 95 | 100 |
| Shrimp | 29 | 5 | 4 | 1 | 19 | 83 | 83 |
| Egg white | 30 | 1 | 4 | 0 | 25 | 100 | 86 |
| Milk | 30 | 1 | 1 | 0 | 28 | 100 | 97 |
| Cockroach | 30 | 6 | 4 | 0 | 20 | 100 | 83 |
| Combined | 149 | 33 | 9 | 6 | 101 | 85 | 92 |

Dp = *Dermatophagoides pteronyssinus*.

shrimp, egg white, milk and cockroach were 0.92, 0.57, 0.63, 0.51 and 0.60, respectively. The correlation coefficient for the 149 pairs was 0.85. The serum Dp-specific IgE levels measured by UniCAP® significantly correlated linearly with the fluorescent intensity generated on the allergen-arrayed chips ($y = 6.928x + 103.6$, $r^2 = 0.8494$) (Fig. 2).

### Comparison of Allergen-Arrayed Chips with UniCAP®

The data of the allergen-chips and the UniCAP® system were both classified into class 0, 1, 2, 3, 4, 5 and 6 based on the specific IgE concentrations. Class 2 and above were considered a positive result in this study. We compared the classes of allergen-specific IgE for Dp, cockroach, shrimp, egg white and milk to assess the sensitivity and specificity. Values of sensitivity and specificity were calculated for allergen-arrayed chips as shown in Table 2. Sensitivity and specificity were more than 82% for the five allergens. Sensitivity of the allergen-arrayed chip for Dp, cockroach, egg white and milk was more than 95%. In addition, the specificity of the allergen-arrayed chip for Dp and milk was more than 97%. In summary, the combined results of sensitivity and specificity of allergen-arrayed chips were 85% and 92%, respectively.

## DISCUSSION

In the present study, multiple allergen-specific IgE were detected by a microarray-based immunofluorescence assay. Initially, various concentrations of commercial semi-purified human IgE were spotted on chips as a reference (Fig. 1). The curve of the fluorescent intensities of the spots showed significant linearity. Such results imply that detection of allergen-specific IgE on allergen-arrayed chips with immunofluorescence assay is feasible. We studied a population of 30 outpatients from our allergy clinic to evaluate the performance of allergen-arrayed chips. The fluorescent intensities of Dp-specific IgE correlated well with the serum Dp-specific IgE detected by the UniCAP® system (Fig. 2).

The Pearson's correlation coefficients for these five allergens were more than 0.50, and the total combined correlation coefficient was 0.85 (Table 1) indicating good correlation between the allergen-arrayed chip and UniCAP®. The

relatively weaker correlation coefficient for milk may be because most of the specific IgE in milk tested by the allergen-arrayed chip and the UniCAP® system were both negative. Therefore, although the results obtained from the allergen-arrayed chip and the UniCAP® for milk were similar, the plotted curve was not significantly linear, and the correlation coefficient was relatively weak.

In clinical use, grading systems are more useful than absolute levels of serum allergen-specific IgE. In this study, 92% of the results were within one class difference between the allergen-arrayed chip and the UniCAP® system. Differences in classes mostly existed in class 0 and 1. Because very low concentrations of specific IgE were detected in many blood samples, small differences in concentration between these two tests may have resulted in differences within classes 0 and 1.

Allergen-arrayed chips had high sensitivity and specificity compared with UniCAP® for the five allergens tested in this study (Table 2). Allergen-arrayed chips had excellent sensitivity and specificity of Dp-specific IgE. Up to 100% sensitivity for egg white, milk, and cockroach as well as specificity of 86%, 97% and 83% respectively suggested good clinical efficacy of allergen-arrayed chips. Different allergen extract purification and detection methods can lead to false positive or false negative results; further evaluation by skin prick tests or Western blot analysis, for example, can help resolve the discrepancy.

The detection of allergen-specific IgE is useful in the diagnosis and management of an IgE-mediated allergic disease. Whenever a causative allergen is determined, patients can do their best to avoid it, and the specific immunotherapy can begin. Thus, a wide spectrum of allergens needs to be tested. The UniCAP® assay proved to be an efficient laboratory system for routine diagnostic testing of allergens; it also provided quantitative results for both diagnosis and initiation of immunotherapy in clinical practice [21]. However, current detection methods, such as UniCAP®, are expensive and require many blood samples to perform a wide spectrum test. Recent developments in allergen-arrayed chips seem to solve these limitations, especially in allergy diagnosis in young children, where a reduced serum volume is of relevance.

Several kinds of allergen microarrays have been developed to detect allergen-specific IgE. Recently, a rolling cycle amplification technique was applied to the microarrays to improve the sensitivity of allergen-specific IgE detection [17,18]. It showed good sensitivity, but the method was more time-consuming and complex. Other allergen chips, which use a spray-type microarrayer, also showed good sensitivity [19]. In this study, a solid-pin microarrayer was applied for precise control of spotting volume and concentration; sensitivity with this method was satisfactory. More recently, an allergen microarray of recombinant allergens was developed to test allergen-specific IgE [22-24]. It allowed for a component-solved diagnosis; however, there is still a wider range of different allergens which need to be tested in microarrays. Many aspects of the allergen-arrayed chip evaluated in this study need to be refined, such as planer surface materials [25], Cy3/Cy5-conjugated streptavidin, detection of fluorescence intensity and the scoring system.

In conclusion, the allergen-arrayed chips and the UniCAP® system seem to provide similar results for clinical diagnosis and treatment. However, the chips are less time-consuming to prepare and analyze, thereby increasing cost-effectiveness, and require minute amounts of sera. In addition, each allergen-arrayed chip contains its own internal calibration curve, which decreases the variability between tests. Quantitative allergen-specific IgE assays on microarray will be possible with further improvements in optimization of surface chemistry, anti-IgE antibody, allergen extract purification and detection scheme.

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# 對過敏病人以過敏原微陣列晶片偵測血清中過敏原特異性免疫球蛋白E之效力

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**目的** 臨床上要判斷過敏病人對何種過敏原過敏，除了依靠詳細的病史詢問及理學檢查之外，過敏原測試可以有助於確認過敏原及進一步的免疫療法，過敏原微陣列晶片是近年新發展出的過敏原測試方法，可以利用少量的血液樣本來偵測血清中的過敏原特異性免疫球蛋白E。本研究嘗試用過敏原微陣列晶片偵測過敏病人血清中過敏原特異性免疫球蛋白E，並評估其效力。

**方法** 以微陣列機將經萃取的過敏原點在經poly-L-lysine處理過的顯微鏡載玻片上，製成過敏原微陣列晶片，採集經臨床診斷有過敏性鼻炎、過敏性氣喘或異位性皮膚炎病人共30人的血液樣本，分別用過敏原微陣列晶片及UniCAP兩種方法偵測血清中屋塵蟎、蟑螂、蛋白、牛奶及蝦等五種過敏原特異性免疫球蛋白E的量及臨床分級，再將兩者結果加以比較。

**結果** 用過敏原微陣列晶片及UniCAP兩種方法偵測血清中這五種過敏原特異性免疫球蛋白E，兩者結果的相關性分別爲0.92、0.60、0.63、0.51及0.57。與UniCAP作比較，過敏原微陣列晶片用以偵測血清中這五種過敏原特異性免疫球蛋白E的敏感度分別爲95%、100%、100%、100%及83%，其特異度分別爲100%、83%、86%、97%及83%。

**結論** 在臨床應用上，過敏原微陣列晶片似乎可以提供與UniCAP類似的結果，而過敏原微陣列晶片可以利用極少量的血液樣本，進行大量而快速的檢驗。（中台灣醫誌 **2004;9:96-102**）

**關鍵詞**

過敏原，過敏原微陣列晶片，過敏原特異性免疫球蛋白E，屋塵蟎

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