Allergen Component Specific IgE Measurement With the Immulite™ 2000 System: Diagnostic Accuracy and Intermethod Comparison

Danilo Villalta,^{1*} Mirella Da Re,¹ Mariaelisabetta Conte,¹ Paola Martelli,¹ Carina Gabriela Uasuf,² Maria Barrale,² Stella Maria La Chiusa,² and Ignazio Brusca²

¹Allergologia e Immunologia Clinica, DML, A.O. “S. Maria degli Angeli”, Pordenone, Italy

²Patologia clinica, Ospedale Buccheri La Ferla, Palermo, Italy

Background: The identification of the allergenic molecules, associated to the advances in the field of recombinant allergens, led to the development of a new concept in allergy diagnosis called component-resolved diagnosis. The aim of our study was to evaluate the diagnostic accuracy of different allergen components using the full automatic singleplex quantitative platform Immulite™ 2000. **Methods:** One hundred ninety-five allergic outpatients (35 to olive pollen, 35 to birch pollen, 35 to profilin, 35 to house dust mites, 35 to peach, and 20 to shrimp) and 20 negative controls were enrolled for the study. Bet v 1, Bet v 2, Ole e 1, Der p 1, Der p 2, Der f 1, Der f 2, Pru p 3, tropomyosin were tested both with Immulite™ 2000 and ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden). **Results:** Sensitivity of allergen-specific Immunoglobulin E (sIgE)

to Ole e 1, Bet v 1, Der p 1, Der p 2, Der f 1, Der f 2, Pen m 1, and Pru p 3 with Immulite™ 2000 was 100%, 100%, 77.1%, 94.3%, 71.4%, 94%, 75%, and 97.1%, respectively, and the specificity was 100% for all the allergens. The overall agreement between Immulite™ 2000 and ImmunoCAP™ (Thermo Fisher Scientific) platforms was 98.6% (Cohen's kappa = 0.979; confidence interval [CI] 95%: 0.960–0.997). From moderate to strong, positive linear correlations between the assays (r^2 from 0.322 to 0.860, and Spearman's rho from 0.824 to 0.971) were showed. **Conclusions:** A high diagnostic accuracy of the sIgE to allergen components measurement with Immulite™ 2000 and a high agreement with ImmunoCAP™ platforms were shown in this study. J. Clin. Lab. Anal. 29:135–141, 2015. © 2014 Wiley Periodicals, Inc.

Key words: component resolved diagnosis; Immulite 2000; allergy diagnosis; molecular allergens

INTRODUCTION

The diagnosis of IgE-mediated allergic disorders is based on both the presence of a compatible clinical history and demonstration of sensitization using in vivo and/or in vitro techniques for the detection of allergen-specific IgE (sIgE). Allergen provocation tests may also be used where necessary.

Until recently, in vitro assays were based on detecting sIgE to total extracts containing a series of allergenic and nonallergenic component. Such tests gave rise to different problems, especially low accuracy due to the difficulty of standardizing the allergens used as substrates, and also the inability to differentiate true cosensitization to different allergenic sources from cross-reactivity (1).

The identification of the individual molecules to which patients are sensitized, associated to the advances in the field of recombinant allergens, led to the development of a new concept in allergy diagnosis, namely, component resolved diagnosis (CRD) (2). With CRD, it is possible to distinguish between patients who are truly allergic to a

*Correspondence to: Danilo Villalta, Dipartimento di Medicina di Laboratorio, Allergologia e Immunologia Clinica, A.O. “S. Maria degli Angeli,” Via Montereale 24, 33170 Pordenone, Italy. E-mail: danilo.villalta@aopn.sanita.fvg.it

Received 18 July 2013; Accepted 8 January 2014

DOI 10.1002/jcla.21741

Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Demographic and Clinical Features of Selected Population

Enrolled patients	n°	Age (years)	M/F ratio	Symptoms
Aeroallergens allergy				
Olive allergy	35	Mean: 29.8; range: 2–74	21/14	$R = 22$; $R + A = 13$
Birch allergy	35	Mean: 39.7; range: 10–68	18/17	$R = 25$; $R + A = 10$
HDM allergy	35	Mean: 33.2; range: 10–64	17/18	$R = 21$; $R + A = 14$
Profilin sensitization	35	Mean: 28.3; range: 6–57	17/18	OAS = 21
Food allergy				
Peach allergy	35	Mean: 22.7; range: 9–42	11/24	OAS = 12; $U = 8$; $U + W = 3$; $U + GI = 4$; Ax = 8
Shrimp allergy	20	Mean: 41.0; range: 20–75	10/10	OAS = 3; $U = 8$; $U + OAS = 3$; $U + R = 1$; $R + A = 2$; Ax = 3

R, rhinitis; A, asthma; OAS, oral allergic syndrome; U, urticaria; W, wheezing; GI, gastrointestinal symptoms; Ax anaphylaxis; HDM, house dust mites.

given biological source from those with cross-reactivity to molecules shared among different biological sources (3,4), to identify the molecules to which specific immunotherapy must be targeted (5), and to predict the potential severity of symptoms in food allergy (6, 7).

Purified natural (n) or recombinant (r) allergens manufactured by gene technology are employed for CRD, either using singleplex or multiplex biochip technology (8). The aim of our study was to evaluate the diagnostic accuracy of different molecules recently released on the market, using the full automatic singleplex quantitative platform Immulite™ 2000 for CRD and compare the results with those obtained with the established ImmunoCAP™. Since the house dust mite (HDM) allergens Der f 1 and Der f 2 are not available on the ImmunoCAP™ platform, we compared the results of HDM molecules with those obtained with ImmunoCAP ISAC™.

MATERIALS AND METHODS

Patients

One hundred ninety-five allergic outpatients and 20 negative controls, who went to the Allergy and Clinical Immunology Unit of Santa Maria degli Angeli Hospital (Pordenone) and Allergy unit of Buccheri La Ferla Hospital (Palermo) from January 2011 to February 2013, were enrolled for the study. One hundred forty patients were hypersensitized to aeroallergens (35 to olive pollen, 35 to birch pollen, 35 to profilin, 35 to HDM) and 55 to food allergens (35 to peach and 20 to shrimp). Peach and olive hypersensitized patients were recruited in Palermo (Sicily, South Italy) where these allergies are more frequent, while the other patients were from Pordenone (in north-eastern Italy). The inclusion criteria were as follows: clinical history consistent with IgE-mediated allergy to the aeroallergens or food allergens considered in the study; both positive skin prick test (SPT) and sIgE to allergen extracts, and (in cases of food allergens) positive challenge test with the relevant allergen when necessary. The exclusion criteria were as follows: sensitization

to cross-reacting plant-food allergens, namely PR-10 and profilin in peach allergic patients, medication that could influence SPT results; and other comorbidities such as autoimmune diseases, immunological disorders, infectious diseases, or cancer. Profilin-hypersensitized patients were selected from patients with pollen allergy, with or without oral allergy syndrome, with positive SPT with palm pollen enriched in profilin and positive sIgE to birch (Bet v 2) and/or grass pollens (Phl p 12) profilin. Negative controls were patients without a clinical history consistent with IgE-mediated allergy and who were negative for SPT and sIgE to all the allergens considered in the study. The demographic and clinical features of the studied population are reported in Table 1. All patients gave an informed consent to blood collection and the study was approved by the local Review Boards.

Skin Tests

SPTs were performed with commercially available allergen extracts (Lofarma, Milan, Italy for olive and birch pollen allergens; ALK-Abellò BV, Nieuwegein, The Netherlands for HDM, shrimp, peach, and palm pollen enriched in profilin). All skin tests were carried out and read following established criteria (9). Only wheals showing a mean diameter exceeding 3 mm at 15' were considered as a positive response. SPT with histamine 10 mg/ml and saline were used as positive and negative controls, respectively.

Serum Samples and In Vitro sIgE Measurement

A serum aliquot of the samples drawn from every subject for the measurement of sIgE using allergen extracts (ImmunoCAP™ 250, Thermo Fisher Scientific) was kept at -20°C until sIgE to allergen components were carried out both with Immulite™ 2000 (Siemens Health Diagnostics, Deerfield, IL) and ImmunoCAP™ 250 platforms, following the manufacturer's instructions. Both platforms have a working range of 0.1–100 kU/l. Since Der f 1 and Der f 2 molecules are not available on the ImmunoCAP™

250 platform, for detection of sIgE to HDM molecules (rDer p 1, rDer p 2 [*Dermatophagoides pteronyssinus*], rDer f 1, rDer f 2 [*Dermatophagoides farinae*]) the ImmunoCAP ISACTM microarray platform (Thermo Fisher Scientific) was used. With ImmuliteTM 2000, the following molecules were tested: nBet v 1 (PR-10 of birch; *Betula verrucosa*); rBet v 2 (profilin of birch; *B. verrucosa*), rMal d 4 (profilin of apple; *Malus domestica*); nOle e 1 (olive; *Olea europea*); nDer p 1, nDer p 2 (HDM; *Dermatophagoides pteronyssinus*), nDer f 1, nDer f 2 (house dust mites; *D. farinae*); nPru p 3 (nonspecific lipid transfer protein [nsLTP] of peach; *Prunus persica*); rPru av 3 (nsLTP of cherry; *Prunus avium*); rPru av 1 (PR-10 of cherry; *P. avium*); rPru av 4 (profilin of cherry; *P. avium*); nPen m 1 (tropomyosin of black tiger shrimp; *Penaeus monodon*).

The following molecules were tested with ImmunoCAPTM 250: rBet v 1 (PR-10 of birch, *B. verrucosa*), rBet v 2 (profilin of birch; *B. verrucosa*), rOle e 1 (olive; *O. europea*), rPru p 3 (nsLTP of peach; *Prunus persica*); rPen a 1 (tropomyosin of brown shrimp; *Penaeus aztecus*).

Statistical Analysis

The number of patients positive for each individual molecule characterizing the different groups of allergy (sensitivity), and the number of positive controls (specificity) was evaluated. A positive result for the quantitative allergen-specific IgE tests was defined as a concentration ≥ 0.35 kU/l.

The results of allergen-sIgE measurement with the ImmuliteTM 2000 and ImmunoCAPTM or ImmunoCAP ISACTM were analyzed to determine intermethod corre-

lation and agreement. Scatter plot, regression analysis, Spearman correlation analysis, and Cohen's kappa analysis for agreement were used, and significance level was set at a *P* value <0.05 . Softwares used in the statistical analysis were SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL) and MedCalc for Windows, version 7.4.9 (MedCalc software, Mariakerke, Belgium).

RESULTS

Sensitivity and specificity of sIgE to the evaluated allergens (Ole e 1, Bet v 1, Der p 1, Der p 2, Der f 1, Der f 2, Pen m 1/Pen a 1, Pru p 3), both with ImmuliteTM 2000 and Thermo Fisher platforms, are shown in Table 2. Very similar data of sensitivity and specificity were obtained using the ImmuliteTM 2000 and Thermo Fisher diagnostic systems.

sIgE to Pru av 1 (PR-10) and Pru av 4 (profilin) were also tested in patients with peach allergy. Their negativity on the one hand confirms the data obtained with ImmunoCAPTM, but on the other hand confirms that all the peach allergic patients have a genuine sensitization to peach and symptoms cannot be attributable to a pollen fruit cross-reactivity. No difference in the accuracy of peach allergy diagnosis has been shown measuring sIgE to nsLTP of peach (Pru p 3) or nsLTP of cherry (Pru av 3), respectively.

Table 3 shows the agreement (%) and Cohen's kappa value for sIgE detection with ImmuliteTM 2000 and the Thermo Fisher platforms. An almost perfect agreement for all the tested allergens was obtained and the overall agreement in a total of 480 paired test resulted 98.6% ($k = 0.979$; 95% CI: 0.960–0.997).

Intermethod comparison of sIgE values was conducted for ImmuliteTM 2000 vs. ImmunoCAPTM that are

TABLE 2. Sensitivity and Specificity of sIgE Measurement to the Individual Molecules Evaluated

Sensitizer	Allergen	Immulite TM 2000		ImmunoCAP TM	
		SE	SP	SE	SP
Olive	Ole e 1	35/35 (100%)	0/20 (100%)	34/35 (97.1%)	0/20 (100%)
Birch	Bet v 1	35/35 (100%)	0/20 (100%)	35/35 (100%)	0/20 (100%)
HDM	Der p 1 ^a	27/35 (77.1%)	0/20 (100%)	27/35 (77.1%)	0/20 (100%)
	Der p 2 ^a	33/35 (94.3%)	0/20 (100%)	32/35 (91.4%)	0/20 (100%)
	Der f 1 ^a	25/35 (71.4%)	0/20 (100%)	25/35 (71.4%)	0/20 (100%)
	Der f 2 ^a	33/35 (94.3%)	0/20 (100%)	31/35 (88.5%)	0/20 (100%)
	Pen m 1/Pen a 1 ^b	15/20 (75.0%)	0/20 (100%)	16/20 (80.0%)	0/20 (100%)
Shrimp					
Peach	Pru p 3 (nsLTP)	34/35 (97.1%)	0/20 (100%)	34/35 (97.1%)	0/20 (100%)
	Pru av 3 (nsLTP)	34/35 (97.1%)	0/20 (100%)	-	-
	Pru av 1 (PR-10)	0/35 (0%)	0/20 (100%)	-	-
	Pru av 4 (profilin)	0/35 (0%)	0/20 (100%)	-	-

SE, sensitivity; SP, specificity.

The specificity has been calculated on the 20 negative controls.

^aSince Der f 1 and Der f 2 molecules are not available in the ImmunoCAPTM 250 platform, for detection of sIgE to house dust mites (HDM) the ImmunoCAPTM ISAC platform has been used.

^bFor detection of sIgE to tropomyosin ImmuliteTM 2000 use Pen m 1 and ImmunoCAPTM Pen a 1 as allergen.

TABLE 3. Agreement Between Immulite™ 2000 and ImmunoCAP™ (ImmunoCAP™ ISAC for HDM Allergens^a) Systems

Tested allergens	Number of tests	Agreement (%)	Kappa (95% CI)
Bet v 1	55	100	1.000 (1.000–1.000)
Ole e 1	55	98.1	0.961 (0.885–1.036)
Der p 1 ^a	55	98.1	0.963 (0.893–1.034)
Der p 2 ^a	55	98.1	0.962 (0.889–1.035)
Der f 1 ^a	55	100	1.000 (1.000–1.000)
Der f 2 ^a	55	98.1	0.963 (0.891–1.034)
Bet v 2	55	100	1.000 (1.000–1.000)
Pen m 1/Pen a 1	40	97.5	0.947 (0.846–1.049)
Pru p 3	55	100	1.000 (1.000–1.000)
Overall	480	98.6	0.979 (0.960–0.997)

Patients selected for each specific-allergen group and negative controls were considered.

methods reporting IgE concentrations in a continuous scale (kU/l) standardized to WHO international Reference Preparation for IgE (second IRP 75/502), and separately for Immulite™ 2000 vs. ImmunoCAP ISAC™, since in the last method the results are expressed as ISAC standardized units (ISU/l), only indirectly linked to WHO IRP 75/502 standard. Immulite™ 2000 yielded median concentration results for sIgE that were higher than those obtained with ImmunoCAP™ or ImmunoCAP ISAC™, for all the allergens tested. Scatterplots of Immulite™ 2000 vs. ImmunoCAP™ and ImmunoCAP ISAC™ are depicted in Figs. 1 and 2, respectively. Regression analysis and Spearman correlation analysis showed moderate to strong positive linear correlation between the assays (Table 4).

Moreover, we compared the results obtained using Immulite™ 2000 to test for sIgE to two different profilins (nBet v 1 and nMal d 4) and two different nsLTP of *Rosaceae* family (nPru p 3 and nPru av 3). The scatterplots are reported in Fig. 3 and regression analysis

between nBet v 2 and nMal d 4 showed a strong linearity ($R^2 = 0.967$; slope 0.987, standard error [SE] 0.033; $P < 0.0001$), as well as between nPru p3 and nPru av 3 ($R^2 = 0.927$; slope 0.930, SE = 0.045, $P < 0.0001$). An almost perfect correlation between nBet v 2 and nMal d 4, and nPru p3 and nPru av 3 was also shown by the Spearman test ($\rho = 0.963$, CI 95%: 0.923–0.892 and 0.960, CI 95%: 0.921–0.980, respectively).

DISCUSSION

ImmunoCAP™ and Immulite™ 2000 are FDA-approved enzyme-linked immunoassays that give quantitative results of sIgE levels. With these diagnostic platforms, it is possible to measure the sIgE level not only to allergen extracts, but also to specific purified or recombinant allergens. In addition to the individual determination of sIgE, it is now possible to simultaneously determine sIgE toward more than 100 allergen components using modern biochip technology (10), with only a very small quantity of serum (ImmunoCAP ISAC™ sIgE 112). The last solid-phase enzyme-linked immunoassay gives semi-quantitative results (ISAC standardized units).

However, until now only Thermo Fisher platforms have been used for CRD in allergy, because only few molecules were available on the Immulite™ 2000. Very recently many new important molecules for CRD on the immulite™ 2000 platform have been released on the market, and in this study we evaluated the diagnostic accuracy of some of these and compared the results with those obtained with the established ImmunoCAP™ or the ImmunoCAP ISAC™ assays.

The specificity of all the evaluated allergens was absolute, since not one of them tested positive in the control population. The sensitivity was of 100% in the selected population with olive and birch allergy, and specifically in olive allergy was higher than the sensitivity obtained with the ImmunoCAP™ system (97.1%). The sensitivity of the individual molecules of HDM ranged from 71.4% of Der

TABLE 4. Regression Analysis and Spearman Correlation Between Immulite™ 2000 and ImmunoCAP™ (^a), and Between Immulite™ 2000 and ImmunoCAP ISAC™ (^b)

Tested allergens	<i>n</i> ^a	<i>R</i> ²	Regression equation	Slope	Spearman's rho (<i>P</i> -value)
Bet v 1 ^a	35	0.791	$y = -3.206 + 0.504x$	0.505	0.971 ($P < 0.0001$)
Bet v 2 ^a	35	0.860	$y = -3.949 + 0.472x$	0.472	0.944 ($P < 0.0001$)
Ole e 1 ^a	35	0.687	$y = -5.542 + 0.639x$	0.639	0.911 ($P < 0.0001$)
Pru p 3 ^a	35	0.647	$y = -5.354 + 0.492x$	0.493	0.955 ($P < 0.0001$)
Pen m 1 ^a	20	0.707	$y = -2.484 + 0.516x$	0.515	0.947 ($P < 0.0001$)
Overall ^a	160	0.714	$y = -4.428 + 0.525x$	0.525	0.946 ($P < 0.0001$)
Der p 1 ^b	35	0.669	$Y = -0.738 + 0.438x$	0.438	0.945 ($P < 0.0001$)
Der p 2 ^b	35	0.322	$Y = -0.890 + 0.267x$	0.267	0.824 ($P < 0.0001$)
Der f 1 ^b	35	0.654	$Y = -1.727 + 0.541x$	0.514	0.964 ($P < 0.0001$)
Der f 2 ^b	35	0.677	$Y = -2.911 + 0.483x$	0.483	0.963 ($P < 0.0001$)
Overall HDM allergens ^b	140	0.530	$Y = -0.949 + 0.386x$	0.386	0.912 ($P < 0.0001$)

Fig. 1. Scatterplot of intermethod comparison between Immulite™ 2000 and ImmunoCAP™ for Ole e 1, Bet v1, Bet v 2, Pru p 3, Pen m 1/ Pen a 1, individually and altogether considered. Dotted line: 95% confidence interval.

p 1 to 94.3% of Der p 2 and Der f 2 and for the combination of all the molecules achieved 97.1%, confirming the results of the study by Weghofer et al. (11), who reported that the combination of Der p 1 and Der p 2 can diagnose $\geq 97\%$ of the *Dermatophagoides pteronissinus* allergic patients in Europe, but not the study of van der Linden et al. (12), who reported for HDM molecules a sensitivity significantly lower than those of the allergen extract. These discrepancies may be attributable to the different selection of the patients. In particular, in our study patients were selected not only for the typical clinical symptoms, but also for positivity of both SPT and sIgE to HDM allergen extract, whereas in the van der Linden study only SPT positivity was a selection factor. The diagnostic sensitivity of sIgE to Der p 2 and Der f 2 measured with Immulite™ 2000 resulted slightly higher than that obtained with ImmunoCAP ISAC™. In contrast, the sensitivity of Immulite™ 2000 for sIgE to tropomyosin was lightly lower than to ImmunoCAP™, but it is important

to note that two different tropomyosin are used in the two assays (from *P. monodon* in the Immulinite™ 2000 and *P. aztecus* in ImmunoCAP™), even if their homology is very high (13). In any case, the sensitivity resulted inferior to the allergen extract and it was expected since tropomyosin is a major allergen of shrimp, but recently other allergenic proteins have been detected, including arginine kinase (14), sarcoplasmic calcium-binding protein (15), and myosin light chain (16), although little is still known about the prevalence of sensitization to these new allergens or about their clinical relevance. Finally, only one patient with peach allergy tested negative for sIgE to nsLTP with both the methods evaluated. Very recently, a new peach allergen (Peamacleins; Pru p 7) has been identified from an Italian group (17), and this may explain the presence of genuine peach allergy negative for sIgE to nsLTP.

The intermethod comparison showed an almost perfect agreement between Immulite™ 2000 and ImmunoCAP

Fig. 2. Fig. 1. Scatterplot of intermethod comparison between Immulite™ 2000 (kU/L) and ImmunoCAP ISAC™ (ISU/L) for Der p 1, Der p 2, Der f 1, and Der f 2, individually and altogether considered. Dotted line: 95% confidence interval.

ISAC™ for all the tested allergens. Regarding the intermethod comparison of sIgE levels between Immulite™ 2000 and ImmunoCAP™, the correlation was discrete to good, even if scatterplot analysis and regression analysis showed as Immulite™ 2000 overestimated all-allergen-specific IgE levels compared with ImmunoCAP™. This result remains difficult to explain since both the methods purport to be standardized to WHO International Reference preparation 75/502 for human IgE. But this result agrees with the results of a previous study by Wang et al.

(18) that compared the levels of sIgE to extract allergen using both the methods.

Even if ImmunoCAP ISAC™ is a semiquantitative method and the results are expressed as ISU and not as kU/L, with the only exception of Der p 2, a good correlation has been shown for HDM allergens between the sIgE levels obtained with this diagnostic platform and Immulite™ 2000. Also, in this case Immulite™ 2000 showed significantly higher sIgE levels, but the absolute values obtained with the two

Fig. 3. Scatterplot of intramethod (Immulite™ 2000) comparison of sIgE to homologous molecules belonging to the profilin (nBet v 2 and nMal d 4) and the nsLTP (nPru p 3 and Pru av 3) families. Dotted line: 95% confidence interval.

diagnostic platforms are not really comparable for the different standardization.

Finally, the intramethod (Immulite™ 2000) comparison between two profilin (nBet v and nMal d 4) and two nsLTP of the *Rosaceae* family (nPru p 3 and nPru av 1) showed a perfect agreement and a very high correlation. So, for the detection of sIgE to profilin in patients with fruit-pollen syndrome and of sIgE to nsLTP in those with allergy to fruits of *Rosaceae* family, the two different molecules of each allergenic class may be used interchangeably.

In conclusion, these results show a very high agreement between the results obtained with Immulite™ 2000 and the established Thermo Fisher platforms for the allergen components evaluated, as well as a good correlation of the IgE levels, even if Immulite™ 2000 overestimated all allergens-specific IgE levels compared with ImmunoCAP™. The increased number of molecules available on the Immulite™ 2000 allows for using this diagnostic platform for CRD in allergy, although the number of molecules is lower than those available on the Thermo Fisher platforms and some important allergen (e.g., grass pollen allergens) are still lacking.

ACKNOWLEDGMENTS

The authors thank Siemens Healthcare Diagnostics for providing the material for sIgE determination free of costs, and Dr. Olindo Ostet for technical assistance.

REFERENCES

1. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;40:1442–1460.
2. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft H, Gronlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
3. Hamilton RG, Franklin AN. In vitro assays for the diagnosis of IgE mediated disorders. *J Allergy Clin Immunol* 2004;114:213–225.
4. Skamstrup Hansen K, Poulsen LK. Component resolved testing for allergic sensitization. *Curr Allergy Asthma Rep* 2010;10:340–348.
5. Sorensen P. The future of specific immunotherapy: Strategies and challenges for the next generation of allergy vaccines. *Allergy* 2011;66(Suppl 95):63–65.
6. Hauser M, Egger M, Wallner M, Wopfner N, Schmidt G, Ferreira F. Molecular properties of plant food allergens: A current classification into protein families. *Open Immunol J* 2008;1:1–12.
7. Hoffmann-Sommergruber K, Mills EN. Food allergen protein families and their structural characteristics and application in component-resolved diagnosis: New data from the EuroPrevall project. *Anal Bioanal Chem* 2009;395:25–35.
8. Treudel R, Simon JC. Overview of component resolved diagnosis. *Curr Allergy Asthma Rep* 2013;13:110–117.
9. Maccario J, Oryszczyn MP, Charpin D, Kauffmann F. Methodologic aspects of the quantification of skin prick test responses: The EGEA study. *J Allergy Clin Immunol* 2003;111:750–756.
10. Lucas JM. Microarrays: Molecular allergology and nanotechnology for personalised medicine. *Allergol Immunopathol (Madr)* 2010;38:153–161.
11. Weghofer M, Thomas WR, Kronqvist M, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 2008;38:959–965.
12. van der Linden IJ, de Groot MJ, de Jong NC, Bozkurt Z, Cobbaert CM. The diagnostic performance of allergen-molecules in comparison to allergen-extracts. *Clin Chem Lab Med* 2011;50:129–132.
13. Jeong KY, Hong CS, Yong TS. Allergenic tropomyosins and their cross-reactivities. *Protein Pept Lett* 2006;13:835–845.
14. Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 2003;170:445–453.
15. Ayuso R, Grishina G, Ibanez MD, et al. Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. *J Allergy Clin Immunol* 2009;124:114–120.
16. Ayuso R, Grishina G, Bardina L, et al. Myosin light chain is a novel shrimp allergen Lit v 3. *J Allergy Clin Immunol* 2008;122:795–802.
17. Tuppo L, Alessandri C, Pomponi D, et al. Peamaclein-a new peach allergenic protein: Similarities, differences and misleading features compared to Pru p 3. *Clin Exp Allergy* 2013;43: 128–140.
18. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. *J Allergy Clin Immunol* 2008;121: 1219–1224.