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A New Method to Bind Allergens for the Measurement of Specific IgE Antibodies

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Allergens · Immunoglobulin E · In vitro tests · Latex · Obeche wood · Occupational medicine · Rye flour · Wheat flour

Abstract
Background: Detection of allergen-specific IgE antibodies in patients' sera plays a key role for the diagnosis of IgE-mediated allergy. If no validated test system is available, diagnostic tools must be developed, usually by coupling or binding the allergens to a solid phase. Streptavidin ImmunoCAP™ is a new solid phase for binding of allergens which can be used in the Pharmacia CAP® system. ***Objective:*** It was the aim of this study to assess the diagnostic validity of Streptavidin ImmunoCAP. ***Methods:*** Biotinylation and allergen concentration for binding to Streptavidin ImmunoCAP were optimized and IgE obtained with natural rubber latex, obeche wood, wheat and rye flour Streptavidin ImmunoCAP were compared with the results of ImmunoCAP™ and Enzyme Allergo-Sorbent Test (EAST) using sera from patients complaining of workplace-related respiratory symptoms. ***Results:*** While the relation of biotin-label and protein was critical (best results were obtained with a 5-fold molar excess), labelled protein for coupling to streptavidin ImmunoCAP was applicable in a wide concentration range. On average, IgE values with streptavidin ImmunoCAP were as high as with ImmunoCAP but considerably higher than values obtained by EAST. ***Conclusion:*** Streptavidin ImmunoCAP is a valuable tool for sensitive and specific measurement of IgE binding to new allergens superior to cellulose disk-based methods.

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Introduction

IgE antibody recognition of allergens is the central event in the pathogenesis of Type I allergy. The recognition of the causal allergens is essential for prevention, diagnosis and immunotherapy. Besides in vivo diagnosis with skin tests and specific allergen challenge, the detection of allergen-specific IgE antibodies (sIgE) in serum plays a key role. To determine the sIgE response to new allergens, researchers have to prepare their own diagnostic tools usually by coupling the allergens to cellulose disks. With the help of this method, alone in the field of occupational allergies several new allergens could be found and investigated in the recent years [1–9]. How-

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ever, IgE measurements based on allergen disks are in disadvantage compared to the ImmunoCAP™ test concerning sensitivity and reproducibility [10–19]. Until recently, the coupling of allergens to ImmunoCAP, the solid phase used for the Pharmacia AutoCAP™ or UniCAP® system, could be performed exclusively by the manufacturer. After Pharmacia developed an experimental prototype of a Streptavidin ImmunoCAP™ as a tool to measure sIgE to unknown or unavailable allergens, we optimized the binding method of allergens to the Streptavidin ImmunoCAP and compared sIgE values with the results of ImmunoCAP and EAST.

Materials and Methods

Subjects

94 sera from patients complaining of workplace-related respiratory symptoms with sIgE concentrations ≥0.35 kU/l according to Pharmacia ImmunoCAP™ (Pharmacia, Freiburg, Germany) to the following occupational allergens: natural rubber latex (NRL; 30 sera), wheat flour (29 sera), rye flour (30 sera), obeche wood (5 sera) were selected. Furthermore, 27 sera from patients (10 health care workers, 9 bakers, 2 chemical workers, 2 metal workers, 1 miller, 1 coal miner, 1 woodworker and 1 biologist) without sIgE to the respective allergens were used as negative controls. 14 control sera had a total IgE level below 100 IU/ml, the remainder ranged between 121 and 772 IU/ml.

Allergen Extracts

Seeds of wheat *(Triticum aestivum)* cultivars Zentos, Bussard, Batis, Contra, Flair, Pegasos, Tarso, Hanseat, Thasos and seeds of rye *(Secale cereale)* cultivars Hacada, Esprit, Halo, Ursus, Petka were crushed, homogenized and extracted for 2 h with 4 parts v/w of phosphate-buffered saline (10 m*M* potassium phosphate pH 7.0, 400 m*M* NaCl, 0.3% phenol). After centrifugation at 20,000 *g*, the pellets were extracted with 70% ethanol (half of the volume of the first extraction) for 1 h at 4°C and centrifuged at 20,000 *g*. Supernatants of all extractions were dialyzed (the salt-soluble fractions with 5 m*M* potassium phosphate and the ethanol-soluble fractions with 1% acetic acid, dialysis cutoff 3.5 kDa) and lyophilized. For allergen preparation, the salt-soluble and ethanol-soluble fractions were blended (7:1).

NRL sap from Malaysia stabilized with glycerin was centrifuged for 30 min at 4°C with 245,000 *g*. The latex C-serum was dialyzed (cutoff 3.5 kDa) and lyophilized.

Obeche wood dust was extracted 16 h at 4°C in 10 parts extraction buffer (50 m*M* sodium phosphate pH 7.4, 150 m*M* NaCl, 2% polyvinylpolypyrrolidone, 0.1 m*M* Pefabloc). The extract was centrifuged at 12,000 *g* at 4°C for 45 min and the supernatant was filtrated, dialyzed (cutoff 3.5 kDa) and lyophilized.

Biotinylation of Proteins

Wheat and rye grain extracts, obeche wood extract and NRL extracts (3.75 mg protein/ml) were biotinylated for 2 h on a rotator at room temperature with a 2.5- to 50-fold molar excess of *D*-biotinoyl-ε-aminocaproic acid N-hydroxysuccinimide ester (NHS-Biotin) in 10 m*M* carbonate buffer (pH 8.5), assuming an average molecular weight of 30 kDa for cereal and latex proteins and 26 kDa for obeche wood proteins. Non-bound biotin label was skipped by gel filtration on PD-10 columns (Pharmacia) and the optical density of the labelled protein was measured at 280 nm (OD_{280} value of 1.5 ≈1 mg protein/ml).

Binding of Labelled Allergens to Streptavidin ImmunoCAP and sIgE Measurements

Prewashed Streptavidin ImmunoCAP (Pharmacia) were loaded with 50 µl labelled protein in varying concentrations, incubated for 30 min, washed and used for sIgE measurements in a Pharmacia CAP system with patients' sera in parallel to ImmunoCAP (f4, f5, k212, k82, batches without recombinant Hev b 5) according to the manufacturer's protocol for sIgE detection. Values of less than 0.35 kU/l represent absent or undetectable levels of allergen-specific IgE antibodies. Values ≥0.35–0.7 kU/l correspond to RAST class 1, values ≥0.7–3.5 kU/l to RAST class 2, values ≥3.5–17.5 kU/l to RAST class 3, values ≥17.5–50 kU/l to RAST class 4; values ≥50–100 kU/l to RAST class 5 and values ≥100 kU/l to RAST class 6.

Coupling of Allergens to Cellulose Disks and Enzyme Allergo-Sorbent Test (EAST)

CNBr-activated cellulose disks were incubated with the allergen extracts in 0.1 *M* $NaHCO_3$ (pH 8.5) containing 1 mg/ml protein for 5 h at room temperature, saturated by ethanolamine and washed according to the method described by Ceska and Lundkvist [20]. The allergen-coated disks were incubated with 50 µl patients' sera, and bound IgE was determined by EAST (Allergopharma, Reinbek, Germany) as described by the manufacturer. The EAST reference curve, always run in parallel, was used for calculation. Detection limits and RAST-classes were the same as described above for ImmunoCAP with the exception that all values ≥17.5 kU/l correspond to RAST class 4.

Data Analysis

Specific IgE values obtained with two different methods were plotted with logarithmic scales on both axes taking into account the approximately log-normally distributed IgE values. For the correlation between the results the squared Pearson correlation coefficients (r^2) of the log-transformed values was calculated.

Results

Optimization of Allergen Binding to Streptavidin ImmunoCAP

For binding of allergens to Streptavidin ImmunoCAP, the protein had to be labelled with biotin. The influence of the relation between NHS-biotin and protein on sIgE results using the Allergen-Streptavidin ImmunoCAP was tested with wheat flour protein and obeche wood protein (table 1). While the relation of biotin-label and protein was critical (highest sIgE results were obtained with a 5-fold molar excess), labelled protein for binding to Streptavidin ImmunoCAP was applicable in a wide concentration range (fig. 1a, b). With cereal protein concentrations from 0.17 to 2.6 mg/ml (OD_{280} 0.25 to 3.95), only minor differences of sIgE values were obtained. Using a lower

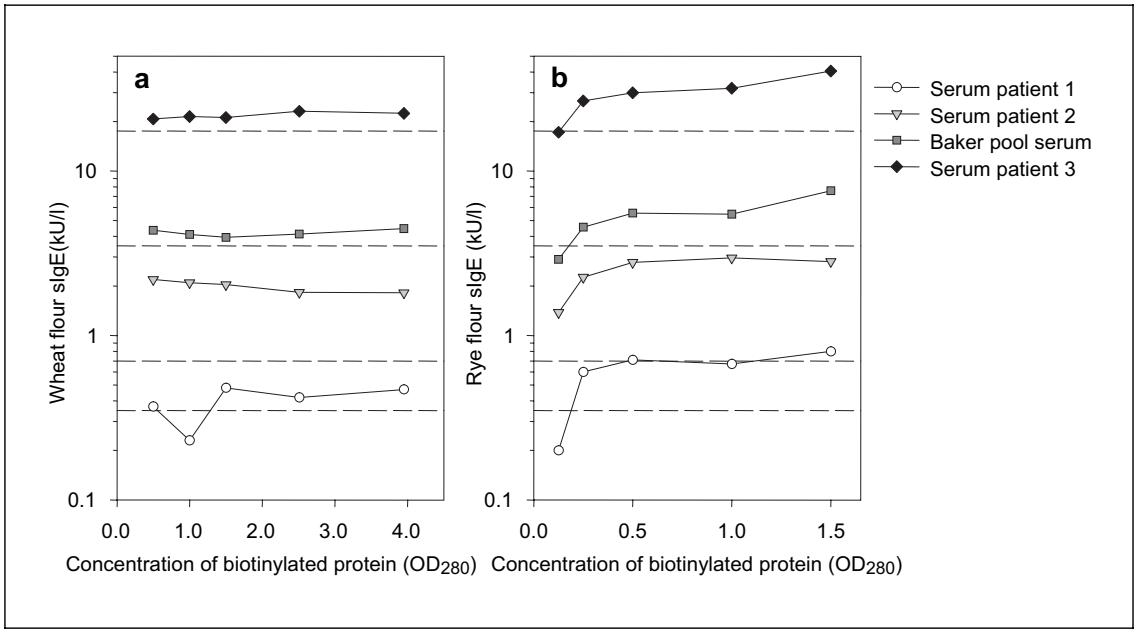

Fig. 1. Influence of protein concentration during binding to Streptavidin ImmunoCAP on sIgE values. Biotinylated wheat flour (**a**) or rye flour (**b**) protein extracts were bound with different protein concentrations (OD 1.5 ≈ 1 mg/ml protein) to Streptavidin ImmunoCAP and sIgE results with a pooled serum and three wheat and rye sensitized patients' sera were measured.

Table 1. Influence of molar excess of biotin label on sIgE results

	sIgE, kU/l (NHS-biotin)				
	2.5-fold	5-fold	10-fold	30-fold	50-fold
Wheat flour					
Miller (negative)	<0.35	<0.35	<0.35	<0.35	<0.35
Baker 1	0.67	0.68	0.48	<0.35	<0.35
Baker 2	2.41	2.76	2.04	1.19	0.86
Baker 3	23.00	27.60	21.10	14.40	13.90
Baker pool serum	4.77	4.97	3.95	2.18	2.01
Obeche wood					
Wood worker (negative)	<0.35	<0.35	<0.35	<0.35	
Wood worker 1	1.46	1.39	1.35	1.22	
Wood worker 2	3.78	5.55	5.25	4.82	
Wood worker 3	21.30	21.70	23.00	16.00	
Wood worker 4	32.80	36.00	27.20	20.80	

Wheat flour and obeche wood protein extracts were coupled with different molar excess of NHS-biotin but the same protein concentration to Streptavidin ImmunoCAP.

protein concentration (≈80 µg/ml rye flour protein, OD_{280} 0.125, fig. 1b) for binding to Streptavidin ImmunoCAP binding rates decreased.

Comparison of Streptavidin ImmunoCAP-Based sIgE Results with ImmunoCAP

There was a close correlation of sIgE concentrations obtained with Allergen-Streptavidin ImmunoCAP and ImmunoCAP (r^2 wheat flour 0.948; r^2 rye flour 0.932; r^2 NRL 0.854; r^2 obeche wood 0.988) (fig. 2). In 50 of 94 cases the ImmunoCAP value was higher than the Streptavidin-ImmunoCAP value, in 41 cases the latter test gave a higher test result, for the rest the results were identical. Sixty-four of 94 tests had identical RAST classes. In 5 cases with positive ImmunoCAP (3 wheat flour, 2 NRL) the Streptavidin ImmunoCAP test was negative, thus sensitivity was 95%. If ImmunoCAP values were compared to previous ImmunoCAP results with the same sera (batch to batch interassay comparison), the coefficients of correlation were in the same range (r^2 wheat flour 0.911, r^2 rye flour 0.907; r^2 NRL 0.981). Additionally, 19 negative control sera (10 from NRL-exposed patients, 9 from patients without professional NRL exposure) were

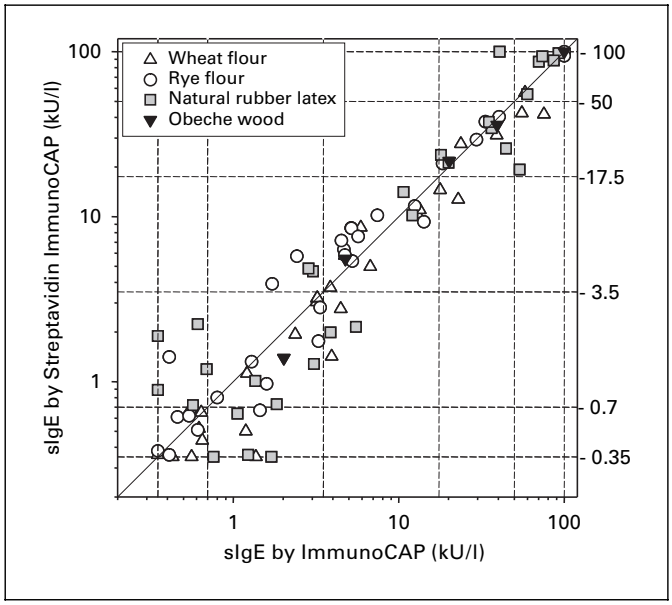

Fig. 2. Comparison of sIgE results obtained by ImmunoCAP for wheat flour, rye flour, NRL and obeche wood and own extracts of these allergens bound to Streptavidin ImmunoCAP (all allergens were labelled with a 5-fold molar excess of NHS-biotin and bound with OD_{280} 1.5). Grid lines indicate limits of RAST-classes.

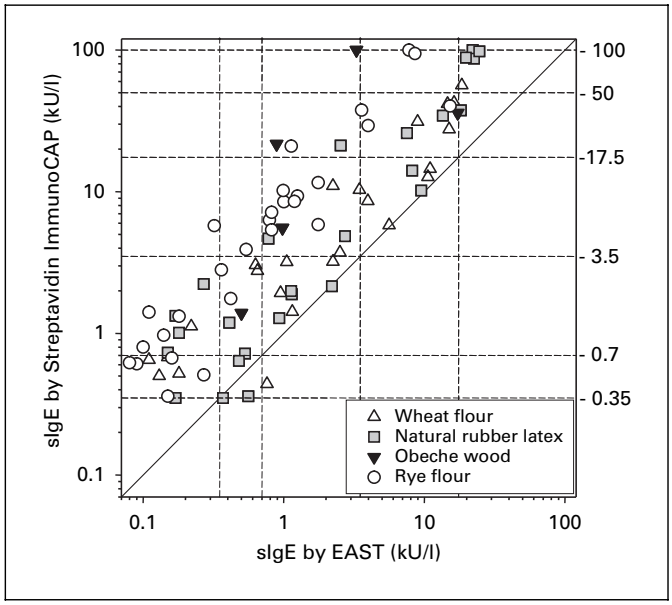

Fig. 3. Comparison of EAST results obtained with own extracts of wheat flour, rye flour, NRL and obeche wood coupled to cellulose disks and CAP results of these allergens bound to Streptavidin ImmunoCAP. Grid lines indicate limits of RAST classes.

tested with NRL-Streptavidin ImmunoCAP, 19 control sera (9 from bakers, 10 from patients with other professions) were tested with wheat or rye flour-Streptavidin ImmunoCAP, and one control serum with Obeche-Streptavidin ImmunoCAP. With the exception of three sera from NRL-exposed patients all control sera gained a negative test result with the Allergen-Streptavidin ImmunoCAP. The three sera showed sIgE values of RAST-class 1 or 2 with the Latex-Streptavidin ImmunoCAP while the k82 ImmunoCAP value was negative. Examination of these sera with rHev b 5 'spiked' k82 ImmunoCAP [21] yielded sIgE concentrations of RAST-class 2 in each case.

Comparison of Streptavidin ImmunoCAP-Based sIgE Results with EAST

EAST results obtained with same allergen extracts and patients' sera were correlated to the Streptavidin ImmunoCAP values (r^2 wheat flour 0.883; r^2 rye flour 0.893, r^2 NRL 0.855, r^2 obeche wood 0.480), but were in 81 from 84 cases lower (fig. 3). Sixty-five of 84 comparisons including all rye flour tests showed lower RAST-classes by EAST, in 20 cases EAST results were below the detection limit resulting in RAST-class 0.

Discussion

For comparison and validation of the Streptavidin ImmunoCAP system, complex occupational allergen sources were selected with commercially available ImmunoCAP diagnostics. NRL [22] and wheat flour [23] have been shown by means of two-dimensional gel electrophoresis to contain more than 200 or 300 distinct polypeptides, and rye flour and obeche wood are also complex protein sources.

Before binding of allergens to Streptavidin ImmunoCAP, the allergens have to be labelled with biotin. For labelling a ≈5-fold molar excess of biotin-label can be used; higher amounts resulted in lower sIgE values most probably as a consequence of masked epitopes. For binding of protein, a wide concentration range was applicable. With protein concentrations from 0.17 to 2.6 mg/ml only minor differences of sIgE values were observed. As a consequence, changes in protein concentration for binding to Streptavidin ImmunoCAP most probably will not result in changes of sIgE values if the protein concentration exceeds a minimum value (OD_{280} of 0.25 for cereal proteins).

The sIgE values obtained with NRL, obeche wood, wheat and rye flour extracts bound to Streptavidin ImmunoCAP were strongly correlated to the ones with k82, k212, f4 and f5 ImmunoCAP and on average of same height although allergen extracts were not identical and the principle of coupling was different: Biotin-Streptavidin binding in one case, covalent coupling to the flexible hydrophilic CNBr-activated cellulose derivative in the other. The differences between test results for cereal flours were within the range of those observed in the batch to batch interassay comparison of ImmunoCAP tests. For NRL, the correlation of NRL-Streptavidin ImmunoCAP and k82 ImmunoCAP was less close than in the k82 batch to batch correlation, indicating a difference in NRL allergen composition. We suspect that the glycerinated Malaysia NRL extract we used for our experiments has a higher content of the allergen Hev b 5 than Pharmacia k82 latex which was found to lack a sufficient amount of this important NRL allergen [21, 24]. That is supported by the fact that three k82 negative sera from latex-exposed patients gave positive results with Latex-Streptavidin ImmunoCAP as well as with 'k82 plus' ImmunoCAP containing rHev b 5 spiked latex. In the account of that, these three cases of divergent sIgE results are no false positives. Then, specificity of Streptavidin ImmunoCAP is 100% in comparison to ImmunoCAP.

The main advantage of the ImmunoCAP in comparison to cellulose disks, the increased surface of the ImmunoCAP which can bind three times as much proteic allergens as the disks seems to take effect also in the Streptavidin ImmunoCAP. The sIgE values of the Streptavidin ImmunoCAP were higher than EAST results in nearly all cases, 77% of tests gave a higher RAST class. In fact, 20 of 84 cases were negative by EAST but positive by Streptavidin ImmunoCAP, resulting in a 24% reduced sensitivity of EAST. Similar differences had been found after the introduction of the Pharmacia CAP system when the results of ImmunoCAP were compared to cellulose disk-based measurements [11, 12]. All studies found an increased sensitivity, higher test results with no or only minor loss of specificity.

As our results with complex occupational allergen mixtures bound to Streptavidin ImmunoCAP show the same improvement compared to EAST, we expect the Streptavidin ImmunoCAP to become a valuable tool for measurement of IgE binding to new or commercially unavailable allergens. Sensitivity and specificity of measurements with Allergen-Streptavidin ImmunoCAP were 95 and 100%, respectively, compared to ImmunoCAP.

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References

1 Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M, Curran AD: Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occup Environ Med 2003; 60:802–804.

2 Baur X, Melching-Kollmuss S, Koops F, Strassburger K, Zober A: IgE-mediated allergy to phytase: A new animal feed additive. Allergy 2002;57:943–945.

3 Merget R, Sander I, Raulf-Heimsoth M, Baur X: Baker's asthma due to xylanase and cellulase without sensitization to alpha-amylase and only weak sensitization to flours. A case study. Int Arch Allergy Immunol 2001;124:502–504.

4 Bossert J, Wahl R: Amaranth: A new allergen in bakeries. Allergologie 2000;23:448–454.

5 Chen Z, Posch A, Cremer R, Raulf-Heimsoth M, Baur X: Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. J Allergy Clin Immunol 1998;102: 476–481.

6 Baur X, Sander I, Posch A, Raulf-Heimsoth M: Baker's asthma due to the enzyme xylanase – a new occupational allergen. Clin Exp Allergy 1998;28:1591–1593.

7 Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE, Baur X: Allergy to *Aspergillus*-derived enzymes in baking industry: Identification of β–xylosidase from *Aspergillus niger* as new allergen (Asp n 14). J Allergy Clin Immunol 1998;102:256–264.

8 Chen Z, Posch A, Lohaus C, Raulf-Heimsoth M, Meyer HE, Baur X: Isolation and identification of hevein as a major IgE-binding polypeptide in Hevea latex. J Allergy Clin Immunol 1997;99:402–409.

9 Park HS, Nahm DH: New occupational allergen in a pharmaceutical industry: serratial peptidase and lysozyme chloride. Ann Allergy Asthma Immunol 1997;78:225–229.

10 Bousquet J, Chanez P, Chanal I, Michel FB: Comparison between RAST and Pharmacia CAP system: A new automated specific IgE assay. J Allergy Clin Immunol 1990;85:1039–1043.

11 Ewan PW, Coote D: Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. Allergy 1990;45:22–29.

12 Pastorello EA, Incorvaia C, Pravettoni V, Bonini S, Canonica GW, Ortolani C, Romagnani S, Tursi A, Zanussi C: A multicentric study on sensitivity and specificity of a new in vitro test for measurement of IgE antibodies. Ann Allergy 1991;67:365–370.

13 Leimgruber A, Mosimann B, Claeys M, Seppey M, Jaccard Y, Aubert V, Peitrequin R, Nisoli MP, Pecoud A: Clinical evaluation of a new in-vitro assay for specific IgE, the immuno CAP system. Clin Exp Allergy 1991;21:127–131.

14 Nishikawa K, Nishikawa M, Teramoto N, Hamamoto N, Ishiguro K: Evaluation of the Pharmacia CAP System, a new test for allergic diagnosis (cedar pollinosis). Auris Nasus Larynx 1992;19:169–174.

15 Jeep S, Kirchhof E, O'Connor A, Kunkel G: Comparison of the Phadebas RAST with the Pharmacia CAP system for insect venom. Allergy 1992;47:212–217.
16 Pastorello EA, Incorvaia C, Pravettoni V, Marelli A, Farioli L, Ghezzi M: Clinical evaluation of CAP System and RAST in the measurement of specific IgE. Allergy 1992;47:463–466.
17 Boccagni P, Favari F, Zanoni G, Pezzini A, Tridente G: Comparison of four in vitro assays for specific IgE detection. Int J Clin Lab Res 1994;24:102–105.
18 Williams PB, Barnes JH, Szeinbach SL, Sullivan TJ: Analytic precision and accuracy of commercial immunoassays for specific IgE: Establishing a standard. J Allergy Clin Immunol 2000;105:1221–1230.
19 Szeinbach SL, Barnes JH, Sullivan TJ, Williams PB: Precision and accuracy of commercial laboratories' ability to classify positive and/or negative allergen-specific IgE results. Ann Allergy Asthma Immunol 2001;86:373–381.
20 Ceska M, Lundkvist U: A new and simple radioimmunoassay method for the determination of IgE. Immunochemistry 1972;9:1021–1030.
21 Lundberg M, Chen Z, Rihs HP, Wrangsjo K: Recombinant spiked allergen extract. Allergy 2001;56:794–795.
22 Posch A, Chen Z, Raulf-Heimsoth M, Baur X: Electrophoretic characterization of latex allergens by two-dimensional electrophoresis. J Allergy Clin Immunol 1996;97:572.
23 Sander I, Flagge A, Merget R, Halder TM, Meyer HE, Baur X: Identification of wheat flour allergens by means of two-dimensional immunoblotting. J Allergy Clin Immunol 2001;107:907–913.
24 Chen Z, Rihs HP, Slater JE, Paupore EJ, Schneider EM, Baur X: The absence of Hev b 5 in capture antigen may cause false-negative results in serologic assays for latex-specific IgE antibodies. J Allergy Clin Immunol 2000;105:S83.