

ORIGINAL ARTICLE

Component-resolved diagnosis from latex allergy by microarray

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Experimental
Allergy

Summary

Background A positive specific IgE (sIgE) result for latex does not always mirror the clinical situation and is frequently found in individuals without overt latex allergy.

Objective We sought to investigate the potential of component-resolved diagnosis (CRD) of latex allergy by microarray and to assess whether the technique allows discriminating genuine allergy from asymptomatic sensitization.

Methods Twenty-six healthy controls without a history of latex allergy with a negative latex sIgE and skin test, 22 latex-allergic patients with a compelling history of latex allergy with a positive latex sIgE and prick test and 20 latex-sensitized individuals with a frequent asymptomatic exposure to natural rubber latex-containing devices with a negative latex skin test but a positive sIgE were also included. CRD was performed with the ImmunoCAP ISAC microarray and traditional singleplexed ImmunoCAP.

Results In all patients, the diagnosis of latex allergy could be established by the combination of recombinant latex components present on the microarray (Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02). Over three-quarters of our patients were sensitized for Hev b 5 and/or Hev b 6.02. Some patients also displayed reactivity for Hev b 1 and/or Hev b 3. In contrast, none of the individuals sensitized to natural rubber latex or control individuals demonstrated IgE reactivity for rHev b 1, rHev b 3, rHev b 5 or rHev b 6.02. Three-quarters of the patients sensitized to latex displayed a positive microarray result for recombinant latex profilin (rHev b 8). In contrast to the results obtained by traditional ImmunoCAP for bromelain, almost no sensitization for cross-reactive carbohydrates was demonstrated by bromelain spotted on the microarray. CRD by traditional singleplexed ImmunoCAP showed highly comparable results.

Conclusion CRD by microarray is a reliable tool for diagnosing latex allergy. In addition, the technique allows discrimination between genuine allergy and sensitization. CRD by microarray can improve the diagnosis of IgE-mediated latex allergy by discriminating between genuine allergy and sensitization. CRD by microarray is a reliable tool to diagnose latex allergy. In addition, the technique allows discrimination between a genuine allergy and simple sensitization.

Keywords basophil activation test, component resolved diagnosis, cross-reactive carbohydrate determinants (CCD), IgE, latex, microarray, profilin

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Introduction

IgE-mediated natural rubber latex (NRL) allergy is an important health problem and a correct diagnosis is an absolute prerequisite for appropriate and potential life-saving management [1, 2]. Generally, physicians rely on quantification of specific IgE (sIgE) and skin tests to confirm their clinical suspicion. However, none of these tests demonstrate an absolute predictive value. On several

occasions, it has been demonstrated that an sIgE result for latex is not always clinically relevant and can be false positive in individuals without overt latex allergy [3–6]. In this regard, it has repeatedly been shown that sensitization to cross-reactive carbohydrate determinants (CCD) and the ubiquitous panallergen profilin from grass and weed pollen or CCD from hymenoptera venom is responsible for significant numbers of positive sIgE results without apparent clinical significance [7–11].

Component-resolved diagnosis (CRD), using microarray technology, has recently been introduced into the field of clinical allergology [12–16]. In contrast to traditional sIgE assays, the technique uses multiple purified natural and recombinant allergen components spotted onto a microarray plate, allowing multiple analyses in a single measurement and to establish individual sensitization profiles. Although the technique seems highly promising, additional comprehensive studies are necessary before it can enter mainstream use.

The primary aim of this study was to assess the accuracy of CRD using microarray technology in the diagnosis of latex allergy and to determine whether the technique allows discrimination between a genuine allergy and asymptomatic sensitization. In addition, we sought to investigate whether this technique can reveal sensitization for complex xylose- and fucose-containing CCD and profilin as mimickers of latex allergy. Results were compared with CRD by traditional singleplexed ImmunoCAP and the outcome of flow-assisted basophil activation tests (BAT).

Materials and methods

Subjects

Twenty-two patients with overt latex allergy were selected [M: 4, F: 18; age 40 years (2–58)]. They all had an evocative history of IgE-mediated latex allergy with urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm or generalized anaphylaxis with hypotension and shock documented by a positive latex sIgE (ImmunoCAP) and skin prick test (SPT) (Stallergènes, Fresnes, France). Twenty NRL-sensitized individuals (M: 8, F: 12; age 26 years (11–54)) defined as being frequently (during professional activity or multiple surgical interventions) but asymptotically exposed to NRL-containing devices with a negative latex SPT (weal and flare 0 mm) but a positive sIgE were included to study whether CRD allows to discriminate between real allergy and sensitization. Finally, 26 non-atopic individuals [M: 8, F: 18; age 22 years (4–56)] without a history of classical inhalant or food allergy frequently exposed to NRL-containing devices without symptoms and a negative latex sIgE served as a control group. The local ethics committee approved the study and patients and controls gave informed consent.

Specific immunoglobulin E: immunoCAP

Quantification of total IgE and sIgE for house dust mite, birch, timothy grass, mugwort, cat, dog, *Cladosporium herbarum*, recombinant NRL components (rHev b 1, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9 and rHev b 11), and two markers for sensitization towards typical complex CCD (containing a β -1,2 xylose and core α -1,3 fucose-containing glycan moiety that is more or less ubiquitous in plant kingdom) (horseradish peroxidase

(HRP) and bromelain (nAna c 2 from pineapple; *Ananas comosus*)) [8, 11] was performed according to the manufacturer's instructions (ImmunoCAP FEIA, Phadia, Brussels, Belgium). A value of allergen-specific IgE < 0.35 kU_a/L was considered negative. The technician who performed analyses of ImmunoCAP, microarray and BAT was unaware of the clinical status.

Specific immunoglobulin E: component resolved diagnosis microarray

We used a commercially available allergen microarray immunoassay (ISACTM, VBC Genomics Bioscience Research, Vienna, Austria) as recommended by the manufacturers and recently described elsewhere [17]. Briefly, microarray reaction sites were incubated with 20 μ L of undiluted patients' serum for 2 h in order to capture allergen-specific IgE antibodies by their corresponding allergen. Subsequently, the microarray slides were rinsed and washed with buffer solution in order to remove unbound serum sIgE. After drying, complexes of allergen-bound specific IgE were stained with a secondary, fluorescence-labelled anti-human IgE for 1 h at room temperature protected from light. After a second rinsing and washing procedure, the fluorescence signals obtained were scanned using a laser scanner (LuxScan 10K, CapitalBio, Beijing, China). Analysis of the corresponding digitized microarray image data was performed with ImmunoCAP ISAC software and image information was transformed into numerical data according to calibration sera of known IgE content. Numerical data were expressed as ISAC Standardized Units (ISU/L).

Skin prick test

SPTs included the afore-mentioned inhalant allergens (HAL, Haarlem, the Netherlands) and Hevea latex (Stallergènes, Stallergènes, Fresnes, France). SPT responses were considered positive only when the weal reaction exceeded 3 mm.

Basophil activation test

Whole blood was primed with an IL-3-containing stimulation buffer. Pre-activated samples were stimulated with serial dilutions (0.01, 0.1, 1 and 10 μ g/mL) of NRL (Stallergènes), anti-IgE (BD Pharmingen, Biosciences, Erembodegem, Belgium) as a positive control or washing solution to measure spontaneous CD63 expression (negative control). To estimate activated basophils, cells were stained with AlexaFluor 488-conjugated monoclonal anti-human IgE (Sigma-Aldrich Chemic GmbH, Sigma-Aldrich, Bernem, Belgium) and monoclonal phycoerythrin-conjugated anti-human CD63 (BD Biosciences) or phycoerythrin-conjugated irrelevant control antibody of identical isotypes (BD Biosciences). Red blood cells were

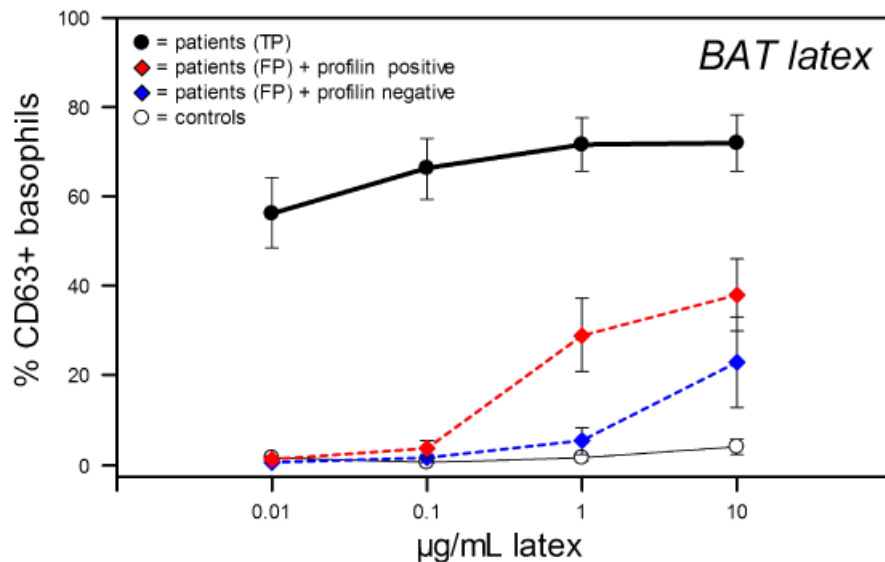


Fig. 1. Latex-induced CD63 up-regulation in healthy control individuals (open circles, $n = 12$), latex-allergic patients (TP, closed circles, $n = 12$), natural rubber latex (NRL)-sensitized individuals not sensitized for profilin (blue diamonds, $n = 7$) and NRL-sensitized individuals sensitized for profilin (red diamonds, $n = 14$). Results are expressed as the percentage of CD63-positive basophils (mean $\pm$ SEM). BAT, basophil activation tests.

lysed and white blood cells were fixed (FACS Lysing Solution, BD Biosciences). Flow cytometric analysis of basophil activation was performed on a FACSCalibur flow cytometer (BD Immunocytometry Systems). Green fluorescence and side scatter were used to characterize the basophils that expressed a high density of surface IgE. Subsequently, within this gate, the percentage of activated basophils co-expressing CD63 was measured. For this purpose, a histogram marker was set on the 99th percentile value obtained with an irrelevant isotype control antibody. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression from the value obtained with allergen stimulation. The preliminary dose-response curves are summarized in Fig. 1. In healthy control individuals, CD63 expression remained almost unaltered. In contrast, a clear CD63 up-regulation above spontaneous expression was observed in latex-allergic patients. Most importantly, from the dose-finding experiments, it was apparent that basophil activation was not restricted to the patients but was also demonstrable in NRL-sensitized individuals, provided the cells were challenged with a higher stimulation concentration. This phenomenon appeared to be particularly relevant for NRL-sensitized individuals with sensitization to profilin. According to the dose-finding experiments and ROC curve analysis, the diagnostic decision threshold was set to 6% for a stimulation concentration of 0.01 $\mu\text{g/mL}$.

Statistical analysis

Results were expressed as median (range). Non-parametric tests were used where appropriate. SPSS 16 software was applied for data analyses.

Results

Total and specific immunoglobulin E

Total IgE in patients, individuals sensitized to NRL and healthy controls was 218 kU/L (15–8950), 550 kU/L (46–4688) and 20 kU/L (1–92), respectively [Kruskal–Wallis (KW) test, $P < 0.001$]. Latex sIgE in the patients was 12.60 kU_a/L (1.10–>100) and 1.50 kU_a/L (0.43–19.60) in the group sensitized for NRL (KW-test, $P < 0.001$).

Basophil activation test

The results of the preliminary dose-finding experiment are summarized in Fig. 1 and discussed above in the method section. Individual percentages of CD63 up-regulation varied from 66 (0–94) in latex-allergic patients to 0 (0–5) in NRL-sensitized individuals, respectively. The percentages of positive BAT in latex-allergic patients and NRL-sensitized individuals are shown in Fig. 2.

Microarray

Table 1 displays the results for all 103 purified and recombinant components tested in the microarray. It is clear that quantification of sIgE by microarray constitutes a specific tool with almost no false-positive results in 26 control individuals without classical inhalant and food allergy. Actually, only 15 positive results (>0 ISU/L) were observed in a total of 2678 quantifications (0.006%) performed in control individuals. Five control individuals showed IgE reactivity for horse serum albumin (Equ c 3,

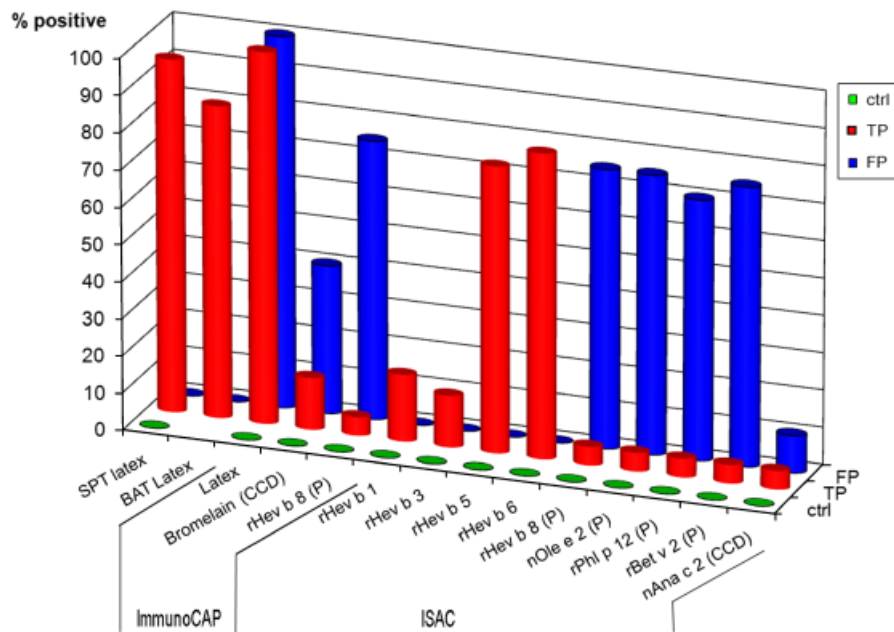


Fig. 2. Percentages of positive skin test (SPT) and sIgE results by ISAC microarray and traditional singleplexed ImmunoCAP in control individuals (CTRL), latex-allergic patients (TP) and individuals sensitized to NRL (false positive: FP). Percentages of positive basophil activation tests (BAT) in TP and FP. Latex allergens: rubber elongation factor (Hev b 1), small rubber particle-associated protein (Hev b 3), acidic latex protein (Hev b 5) and hevein (Hev b 6). Profilins (P): latex (Hev b 8), olive (Ole e 2), timothy grass (Phl p 12), birch (Bet v 2). Recombinant proteins are designated by "r". nAna c 2 is bromelain purified from pineapple (*Ananas comosus*) and is used as an indicator for sensitization for cross-reactive carbohydrate determinants (CCD).

Equus caballus), a 65 kDa allergen that is also demonstrable in horse sweat [18, 19].

All patients with latex allergy had their diagnosis documented by the panel of recombinant components spotted on the microarray (no false-negative results). Figure 2 reveals that over three-quarters of our patients are sensitized for latex acidic protein (Hev b 5) and/or hevein (Hev b 6.02). Some patients also display reactivity for the rubber elongation factor (Hev b 1) and the small rubber particle-associated allergen (Hev b 3).

In contrast, none of the individuals sensitized to NRL or healthy control individuals demonstrate IgE reactivity for microarrayed rHev b 1, rHev b 3, rHev b 5 and rHev b 6.02 (Fig. 2). However, three-quarters of the patients sensitized to NRL display a positive microarray result for recombinant latex profilin (rHev b 8) (Fig. 2), which is highly correlated with other profilins such as purified profilin nOle e 2 from olive (*Olea europaea*) and recombinant profilin rPhl p 12 from timothy grass (*Phleum pratense*) and rBet v 2 from birch (*Betula verrucosa*) (Fig. 3). On the other hand, there is no apparent sensitization for CCD as assessed by the microarray as almost no IgE reactivity for purified bromelain (nAna c 2) was demonstrated (Table 1 and Fig. 2). However, seven out of the 20 individuals sensitized to NRL demonstrate a positive microarray result for the purified glycosylated major allergen from olive Ole e 1 (*Olea europaea*), a tree that does not grow in our regions.

Table 1 shows that NRL-sensitized individuals were almost invariably sensitized for grass pollen as revealed by the sensitization for different genuine recombinant antigens of timothy grass such as rPhl p 1, rPhl p 2, rPhl p 4, rPhl p 5 and rPhl p 6 and the purified major allergen Cyn d 1 from Bermuda grass (*Cynodon dactylon*). Thirteen of these individuals also demonstrate sensitization for tree pollen with a positive microarray result for rBet v 1, the major birch allergen. Sensitization towards weed pollen, as assessed through a positive microarray result for nArt v 1, nAmb a 1 and nSal k 1, the major allergen from mugwort (*Artemisia vulgaris*), ambrosia (*Ambrosia artemisiifolia*) and Russian thistle (*Salsola kali*) was rare.

Comparison microarray with ImmunoCAP

In 13 patients with latex allergy, CRD was also performed with traditional singleplexed ImmunoCAP. This analysis implied quantification of sIgE for the genuine latex components loaded on the microarray (rHev b 1, rHev b 3, rHev b 5 and rHev b 6.02) in addition to rHev b 6.01 (prohevein), rHev b 9 (enolase) and rHev b 11 (class 1 chitinase) (Fig. 4). Data obtained by traditional singleplexed ImmunoCAP confirm that the majority of our patients with latex allergy are sensitized to Hev b 5 and/or Hev b 6.02 and that only some patients react to rHev b 1 and rHev b 3. Six patients

Table 1. Results expressed as median (range) for all 103 purified and recombinant components tested in the microarray in control individuals (controls, $n = 26$), latex allergic patients (patients, $n = 22$), and individuals sensitized for natural rubber latex (false positives, $n = 20$)

	Controls ($n = 26$)			Patients ($n = 22$)			False positives ($n = 20$)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
Plants									
Primarily genuine markers									
nAct d 1	–	–	–	1	0.35	–	3	0.82	(0.33–1.09)
nAct d 2	–	–	–	–	–	–	4	2.94	(0.99–22.96)
nAct d 5	–	–	–	–	–	–	1	1.04	–
rBer e 1	–	–	–	–	–	–	–	–	–
rAna o 2	–	–	–	–	–	–	–	–	–
nCor a 9	–	–	–	1	0.58	–	–	–	–
nSes i 1	–	–	–	1	0.55	–	1	0.22	–
nAra h 1	–	–	–	–	–	–	1	0.94	–
nAra h 2	–	–	–	–	–	–	–	–	–
nAra h 3	–	–	–	–	–	–	–	–	–
nGly m β -conglycinin	–	–	–	1	1.33	–	–	–	–
nGly m glycinin	–	–	–	1	0.51	–	–	–	–
nTri a 18	–	–	–	3	0.22	(0.16–0.43)	–	–	–
rTri a 19.0101	–	–	–	–	–	–	–	–	–
nTri a aA_TI	–	–	–	–	–	–	–	–	–
nTri a Gliadin	–	–	–	1	0.25	–	–	–	–
nCyn d 1	–	–	–	5	20.23	(0.32–33.24)	19	5.41	(0.43–25.56)
rPhl p 1	2	0.54	(0.54–0.54)	9	3.96	(0.12–61.29)	19	17.48	(0.28–66.88)
rPhl p 2	–	–	–	3	42.96	(24.46–49.59)	14	10.145	(0.95–62.44)
rPhl p 4	2	0.25	(0.25–0.25)	4	1.205	(0.61–47.52)	19	9.53	(0.44–40.95)
rPhl p 5	1	0.31	–	5	31.05	(0.23–55.35)	16	22.435	(1.13–71.43)
r'Phl p 6	–	–	–	4	6.555	(2.07–41.67)	13	2.72	(0.75–21.30)
rPhl p 11	–	–	–	3	21.77	(1.51–33.42)	4	2	(1.16–5.21)
nOle e 1	1	0.79	–	–	–	–	7	5.35	(1.31–30.14)
rPla a 1	–	–	–	2	0.155	(0.12–0.19)	–	–	–
nPla a 2	–	–	–	5	0.22	(0.18–0.92)	7	0.61	(0.46–1.38)
nCry j 1	–	–	–	–	–	–	5	0.46	(0.20–11.02)
nCup a 1	–	–	–	–	–	–	3	1.09	(0.38–18.53)
nAmb a 1	–	–	–	–	–	–	–	–	–
nArt v 1	–	–	–	–	–	–	3	3.6	(2.22–8.90)
nSal k 1	1	4.7	–	1	0.37	–	1	1.64	–
rHev b 1	–	–	–	4	2.03	(1.57–4.25)	–	–	–
r'Hev b 3	–	–	–	3	1.66	(0.31–4.21)	–	–	–
rHev b 5	–	–	–	17	16.31	(1.86–66.66)	–	–	–
rHev b 6	–	–	–	18	7.305	(0.30–52.45)	–	–	–
Cross-reactive markers									
PR-10/Bet v 1 homologues									
rBet v 1	–	–	–	6	24.055	(0.60–71.73)	13	30.16	(7.37–66.10)
rAln g 1	–	–	–	5	4.05	(2.29–17.7)	13	8.45	(1.72–55.97)
rCor a 10101	–	–	–	4	5.25	(1.51–19.69)	13	5.62	(0.88–36.86)
rCor a 10401	–	–	–	5	1.64	(1.41–25.13)	13	4.71	(0.56–44.89)
rMal d 1	–	–	–	4	2.52	(0.97–7.43)	13	5.86	(0.47–43.04)
r'Pru p 1	–	–	–	6	2.575	(0.07–8.80)	13	4.5	(0.72–47.89)
rGly m 4	–	–	–	1	5.87	–	10	2.095	(0.49–12.57)
rAra h 8	–	–	–	4	5.035	(0.30–10.73)	12	3.15	(0.16–13.57)
rApi g 1	–	–	–	–	–	–	6	3.89	(1.67–10.24)
rDau c 1	–	–	–	–	–	–	5	0.95	(0.62–1.22)
nAct d 8	–	–	–	1	7.86	–	4	0.88	(0.32–7.5)
Lipid transfer proteins									
nPru p 3	–	–	–	1	1.94	–	3	1.15	(1.08–1.81)
rCor a 8	–	–	–	–	–	–	–	–	–
nArt v 3	–	–	–	–	–	–	3	1.28	(1.02–4.67)

Table 1. continued

	Controls (<i>n</i> = 26)			Patients (<i>n</i> = 22)			False positives (<i>n</i> = 20)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
rPar j 2	–		–	1	0.55		–		–
Profilins									
rBet v 2	–		–	1	48.8		15	4.55	(1.54–39.05)
nOle c 2	–		–	1	42.26	–	15	5.34	(1.43–34.09)
rHev b 8	–		–	1	47.82	(47.82–47.82)	15	7.08	(1.20–49.25)
rMer a 1	–		–	1	50.87	–	15	7	(1.20–46.48)
rPhl p 12	–		–	1	14.1	–	14	2.765	(0.75–32.7)
Calcium binding 2-EF-hand proteins									
rBet v 4	–		–	–		–	3	10.07	(0.23–18.16)
rPhl p 7	–		–	–		–	2	14.905	(10.03–19.78)
CCD marker									
nAna c 2	–		–	1	5.01	–	2	0.9	(0.27–1.53)
Non-plants									
Primarily genuine markers									
nGal d 1	–		–	2	0.265	(0.26–0.27)	–		–
nGal d 2	–		–	1	0.42	–	–		–
nGal d 3	–		–	–		–	–		–
nBos d 4	–		–	–		–	–		–
nBos d 5	–		–	1	1.41	–	–		–
nBos d 8	–		–	1	2.71	(2.71–2.71)	–		–
nBos d lactoferrin	1	0.55	–	3	1	(0.39–2.14)	3	0.27	(0.22–0.45)
rBla g 1	1	0.56	–	–		–	–		–
rBla g 2	–		–	2	0.22	(0.10–0.34)	–		–
rBla g 4	–		–	2	0.195	(0.12–0.27)	–		–
rBla g 5	–		–	–		–	–		–
rFel d 1	–		–	7	26.08	(0.60–57.28)	8	10.26	(0.95–35.67)
rFel d 4	–		–	3	0.45	(0.31–49.42)	1	0.22	–
rCan f 1	–		–	6	14.135	(2.70–52.7)	3	5.22	(1.34–12.05)
rCan f 2	–		–	4	12.965	(2.84–52.34)	–		–
nMus m 1	–		–	1	37.88	–	–		–
rAlt a 1	–		–	–		–	4	19.355	(8.19–32.28)
rAlt a 6	–		–	3	0.3	(0.21–2.43)	–		–
rAsp f 1	–		–	1	0.25	–	1	0.3	–
rAsp f 2	–		–	–		–	–		–
rAsp f 3	–		–	–		–	1	0.72	–
rAsp f 4	–		–	–		–	–		–
rAsp f 6	–		–	2	1.905	(0.76–3.05)	1	10.35	–
rCla h 8	–		–	1	0.35	–	1	0.15	–
rAni s 1	1	0.8	–	–		–	–		–
nApi m 1	–		–	1	2.45	–	1	2.38	–
nApi m 4	–		–	–		–	2	0.81	(0.34–1.28)
Cross-reactive markers									
Cysteine protease									
nDer p 1	–		–	7	9.65	(0.34–51.65)	6	17.185	(1.62–43.74)
nDer f 1	–		–	7	9.53	(0.35–51.09)	6	10.595	(1.18–42.63)
NPC2 family									
rDer f 2	–		–	7	44.61	(2.83–54.51)	8	13.695	(0.32–66.85)
nDer p 2	–		–	7	45.23	(4.09–58.57)	8	12.885	(0.33–66.56)
rEur m 2	–		–	7	7.34	(0.50–31.17)	6	6.81	(0.42–9.95)
Parvalbumin									
rCyp c 1	–		–	3	0.45	(0.35–29.08)	–		–
rGad c 1	–		–	3	0.57	(0.29–41.62)	–		–
Tropomyosin									
rPen a 1	–		–	6	0.235	(0.12–0.76)	–		–

Table 1. continued

	Controls (<i>n</i> = 26)			Patients (<i>n</i> = 22)			False positives (<i>n</i> = 20)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
nPen i 1	–	–	–	2	0.71	(0.20–1.22)	–	–	–
nPen m 1	–	–	–	2	1.025	(0.24–1.81)	–	–	–
rDer p 10	–	–	–	3	0.68	(0.36–2.62)	–	–	–
nBla g 7	–	–	–	1	3.3	–	–	–	–
rAni s 3	–	–	–	1	1.9	–	1	0.23	–
Serum albumin									
nBos d 6	–	–	–	1	0.51	–	–	–	–
nFel d 2	–	–	–	1	1.93	–	–	–	–
nCan f 3	–	–	–	2	1.36	(1.20–1.52)	–	–	–
nEqu c 3	5	0.93	(0.24–3.77)	2	0.435	(0.37–0.5)	–	–	–
nGal d 5	–	–	–	–	–	–	–	–	–

N indicates the number of positive quantifications.

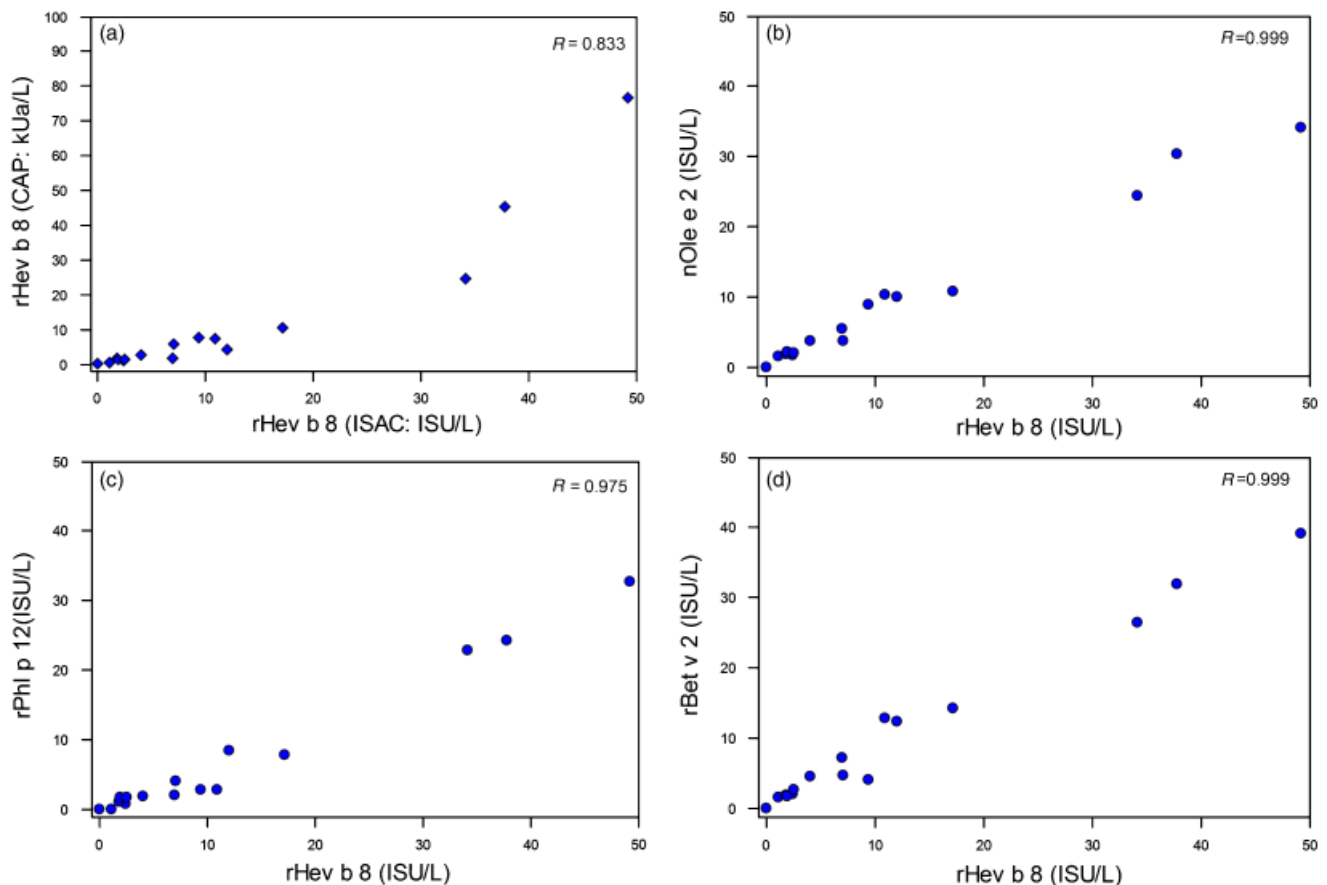


Fig. 3. Correlation between recombinant latex profilin (rHev b 8) by ImmunoCAP and microarray (a). Correlation between latex profilin and purified profilin nOle e 2 from olive (*Olea europaea*) (b), recombinant profilin rPhl p 12 from timothy grass (*Phleum pratense*) (c) and rBet v 2 from birch (*Betula verrucosa*) (d).

(46%) react to rHev b 11. As in other series [11], rHev b 11 was only recognized in sera reactive to rHev b 6.01, probably reflecting the extensive homology between the hevein domain of Hev b 6 and Hev b 11 [20]. No reactivity was observed for rHev b 9. Moreover, as shown in Fig. 5,

latex sIgE, as quantified by traditional ImmunoCAP, was related to sIgE for rHev b 5 and rHev b 6.02 by the microarray technique. Figure 5 also shows the correlation between the microarray and the traditional technique for rHev b 5 and rHev b 6.02.

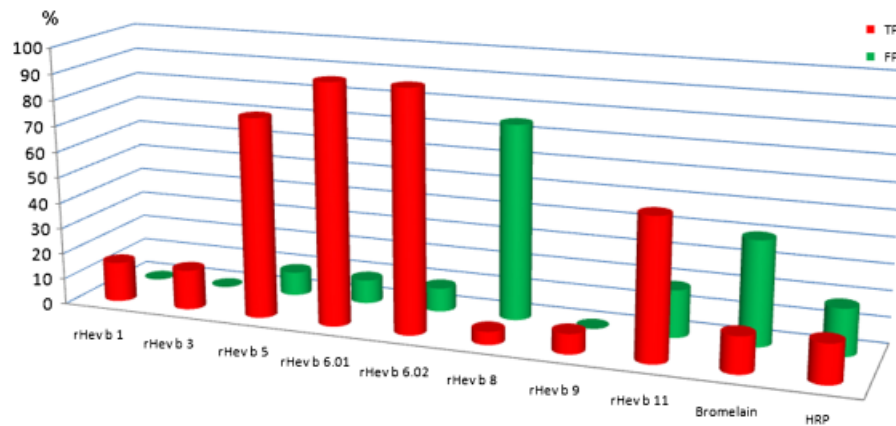


Fig. 4. Percentages of positive sIgE results for genuine and cross-reactive latex components, bromelain and horseradish peroxidase (HRP) by traditional singleplexed ImmunoCAP in patients (TP) and individuals sensitized to natural rubber latex (FP).

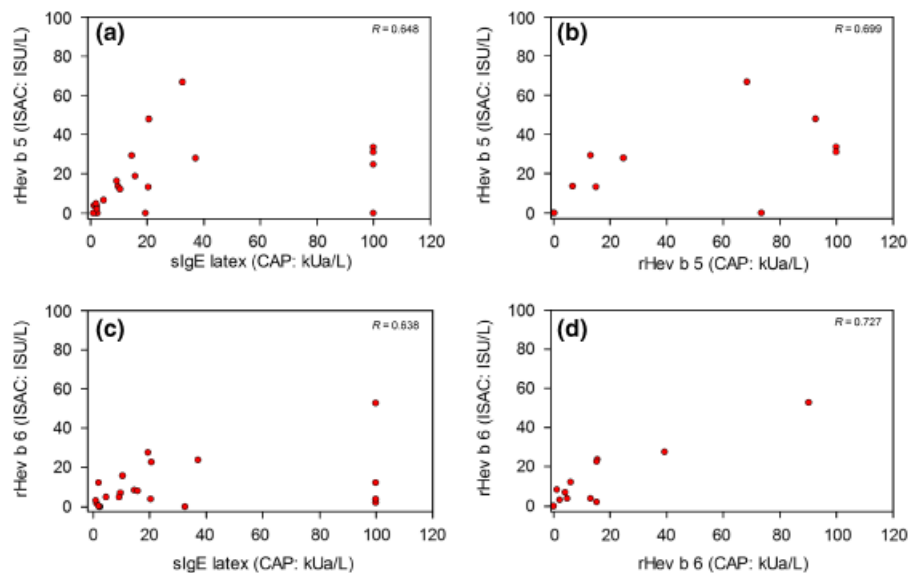


Fig. 5. Correlation between sIgE latex by ImmunoCAP and rHev b 5 by microarray (a), specific IgE (sIgE) rHev b 5 by ImmunoCAP and rHev b 5 by microarray (b), sIgE latex by ImmunoCAP and rHev b 6 by microarray (c), sIgE rHev b 6 by ImmunoCAP and rHev b 6 by microarray (d).

CRD with traditional singleplexed ImmunoCAP also involved 11 individuals sensitized to NRL. Except for one sample (total IgE over 2600 kU/L) that displayed low reactivity for rHev b 5, rHev 6.01 and rHev b 6.02, all quantifications were negative (<0.35 kU_a/L).

The impact of profilin, as demonstrated by the microarray, on the diagnosis of latex allergy was confirmed by traditional ImmunoCAP. Specific IgE for latex profilin (rHev b 8) was <0.35 kU_a/L (<0.35 –78.60) in the patients and 1.64 kU_a/L (<0.35 –76.60) in the individuals sensitized to NRL, respectively ($P < 0.001$). The percentages of positive sIgE results for rHev b 8 are summarized in Figs 2 and 4. Figures 2 and 4 also display the percentage of positive sIgE results for the CCD markers bromelain and HRP. Whereas only three of the 22 (13.6%) sera of latex-allergic patients contained sIgE that reacted to bromelain, eight of the 20

(40%) of the sera from NRL-sensitized individuals were positive for this CCD marker. Only two sera from latex-allergic patients and two sera from individuals sensitized to NRL displayed low anti-HRP concentrations (data not shown). There was no correlation between the traditional bromelain ImmunoCAP and nOle 1 on the microarray.

Discussion

Currently the diagnosis of latex allergy relies on an evocative history along with the demonstration of latex-specific serum IgE antibodies or a positive latex skin test. However, as already addressed in the introductory paragraph, it has been repeatedly shown that these tests do not have an absolute predictive value. A positive sIgE result

for latex does not always mirror the clinical situation and is frequently found in individuals without apparent latex allergy. The major cause for these "false-positive" latex sIgE results seems to be sensitization against ubiquitous structures such as β -1,2 xylose and core α -1,3 fucose-containing cross-reactive CCD and the panallergen profilin. Therefore, additional reliable tests would be more than welcome. This study confirms that the diagnosis of latex allergy can benefit from flow-assisted analysis of *in vitro* activated basophils, as the technique generally allows discrimination between real allergy and sensitization [21]. However, our data suggest that NRL-induced basophil activation was not restricted to the patients. Basophil activation was also demonstrable in NRL-sensitized individuals, particularly these sensitized to profilin, provided the cells were challenged with higher stimulation concentrations. The exact reason(s) for this finding and the observation profilin-sensitized individuals to demonstrate negative skin tests for latex remain(s) elusive. Although purely speculative, a potential explanation could be the scarce presence of profilin in NRL extracts for BAT and skin testing [22, 23] and only sufficient to induce effector cell activation when a higher stimulation concentration is applied. Anyhow, it is fair to state that there is room for new tests that are more readily accessible and that might further elucidate the clinical outcome of particular sensitization profiles.

Our data confirm that the diagnosis of latex allergy can correctly be established by CRD microarray technology and that results of the new microarray technique are comparable to the data obtained by CRD through traditional singleplexed sIgE assays. In our patients, latex allergy generally results from sensitization to the acidic latex protein (Hev b 5) and hevein (Hev b 6.02), both well-known major allergens from *Hevea brasiliensis* (natural rubber tree) [11, 24, 25]. Some patients also demonstrate sensitization to the rubber elongation factor (Hev b 1) and/or the small rubber particle-associated protein (Hev b 3). If combined rHev b 5, rHev b 6.02, rHev b 3 and rHev b 1 together, the microarray-diagnosed latex allergy in all our patients. This observation parallels the findings from other CRD studies that have demonstrated that panels including Hev b 5, Hev b 6 and Hev b 1 generally allow to identify the majority of latex-allergic patients [11, 25–30]. However, as recently addressed in this Journal, it should be kept in mind that the panel of recombinant NRL components currently spotted on the chip does not cover the entire spectrum of the latex IgE response and that additional components such as the minor allergens Hev b 2 and Hev b 13 [31] might be necessary to establish a correct diagnosis in particular cases [11]. Moreover, using a broader repertoire of components might also in evaluating cross-reactivity and identifying patients at risk for a latex-fruit syndrome [32]. Unfortunately, Hev b 2 and Hev b 13 are currently not readily available for *in vitro* diagnosis.

A second purpose of this study was to establish whether CRD could discriminate between real allergy and sensitization. For this purpose, an additional comparative analysis was performed with individuals sensitized to NRL. Although we cannot exclude that pre-selection of atopic individuals with a negative skin test might have introduced a selection bias, this does not seem to be a major issue, as a clinically irrelevant positive skin test for latex appears to be a rare observation [30]. Moreover, it is our experience that patients with an apparently clinically irrelevant positive skin test can hardly be considered as simply sensitized, as they generally demonstrate a positive sIgE and BAT for latex. We consider them at risk and advocate latex preventive measures in those cases. Anyhow, from this additional analysis it appears that sera from individuals sensitized to NRL display no reactivity for Hev b 6.02, Hev b 5, Hev b 3 or Hev b 1 in the microarray. This observation is largely confirmed by the analyses performed with the traditional singleplexed assay. Moreover, our data confirm that apparently irrelevant serum sIgE antibodies against latex generally result from sensitization for grass pollen profilin and/or CCD and that recognition of these components can improve the diagnosis of latex allergy [7–10, 22, 33, 34]. In this context, it is noteworthy that the microarray confirms the large extent of cross-reactivity among plant profilins (and thus allowing the use of a single profilin for diagnosis) [10], but generally fails to detect sensitization for CCD as revealed by positive results for bromelain by a traditional sIgE assay. The exact reason(s) for the discrepancy between the microarray and the traditional singleplexed ImmunoCAP remain(s) elusive. However, it seems theoretically justified to assume that the differences concerning CCD recognition between the two assays might reflect a different presentation of the carbohydrate epitopes [35]. Anyhow, our data suggest that testing for CCD should not be restricted to purified bromelain but should involve different CCD markers. Whether the purified CCD-bearing major allergen from olive (nOle 1) [36] constitutes a candidate CCD marker remains to be further established.

In conclusion, CRD both by microarray and by singleplexed ImmunoCAP seems to be a reliable tool to diagnose latex allergy. In addition, the technique allows discrimination between clinically relevant and irrelevant sIgE results that result from sensitization to profilins. However, it appears that the microarray technique fails to identify sensitization for CCD as revealed by the traditional ImmunoCAP for bromelain. We anticipate that additional and alternative components (including a cocktail of CCD markers) will further optimize the performance of the novel assay.

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References

- Pumphrey RS, Duddridge M, Norton J. Fatal latex allergy. *J Allergy Clin Immunol* 2001; **107**:558.
- Turillazzi E, Greco P, Neri M, Pomara C, Riezzo I, Fineschi V. Anaphylactic latex reaction during anaesthesia: the silent culprit in a fatal case. *Forensic Sci Int* 2008; **179**:e5–e8.
- Ebo DG, Stevens WJ, Bridts CH, De Clerck LS. Latex-specific IgE, skin testing, and lymphocyte transformation to latex in latex allergy. *J Allergy Clin Immunol* 1997; **100**:618–23.
- Biagini RE, Krieg EF, Pinkerton LE, Hamilton RG. Receiver operating characteristics analyses of Food and Drug Administration-cleared serological assays for natural rubber latex-specific immunoglobulin E antibody. *Clin Diagn Lab Immunol* 2001; **8**:1145–9.
- Hamilton RG, Peterson EL, Ownby DR. Clinical and laboratory-based methods in the diagnosis of natural rubber latex allergy. *J Allergy Clin Immunol* 2002; **110**:S47–56.
- Biagini RE, MacKenzie BA, Sammons DL *et al*. Latex specific IgE: performance characteristics of the IMMULITE 2000 3gAllergy assay compared with skin testing. *Ann Allergy Asthma Immunol* 2006; **97**:196–202.
- Aalberse RC, Akkerdaas J, van RR. Cross-reactivity of IgE antibodies to allergens. *Allergy* 2001; **56**:478–90.
- Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy* 2004; **34**:137–44.
- Malandain H, Giroux F, Cano Y. The influence of carbohydrate structures present in common allergen sources on specific IgE results. *Eur Ann Allergy Clin Immunol* 2007; **39**:216–20.
- Radauer C, Willeroider M, Fuchs H *et al*. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006; **36**:920–9.
- Raulf-Heimsoth M, Rihs HP, Rozynek P *et al*. Quantitative analysis of immunoglobulin E reactivity profiles in patients allergic or sensitized to natural rubber latex (*Hevea brasiliensis*). *Clin Exp Allergy* 2007; **37**:1657–67.
- Hiller R, Laffer S, Harwanegg C *et al*. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; **16**:414–6.
- Jahn-Schmid B, Harwanegg C, Hiller R *et al*. Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003; **33**:1443–9.
- Wohrl S, Vigl K, Zehetmayer S *et al*. The performance of a component-based allergen-microarray in clinical practice. *Allergy* 2006; **61**:633–9.
- Harwanegg C, Hutter S, Hiller R. Allergen microarrays for the diagnosis of specific IgE against components of cow's milk and hen's egg in a multiplex biochip-based immunoassay. *Methods Mol Biol* 2007; **385**:145–57.
- Ott H, Baron JM, Heise R *et al*. Clinical usefulness of microarray-based IgE detection in children with suspected food allergy. *Allergy* 2008; **63**:1521–8.
- Deinhofer K, Sevcik H, Balic N *et al*. Microarrayed allergens for IgE profiling. *Methods* 2004; **32**:249–54.
- Goubran BH, Gregoire C, Rabillon J, David B, Dandeu JP. Cross-antigenicity of horse serum albumin with dog and cat albumins: study of three short peptides with significant inhibitory activity towards specific human IgE and IgG antibodies. *Immunology* 1996; **88**:340–7.
- Goubran BH, Rabillon J, Gregoire C, David B, Dandeu JP. Thiophilic adsorption chromatography: purification of Equ c2 and Equ c3, two horse allergens from horse sweat. *J Chromatogr B Biomed Sci Appl* 1998; **710**:57–65.
- O'Riordain G, Radauer C, Hoffmann-Sommergruber K *et al*. Cloning and molecular characterization of the *Hevea brasiliensis* allergen Hev b 11, a class I chitinase. *Clin Exp Allergy* 2002; **32**:455–62.
- Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ. Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy* 2002; **57**:706–12.
- Vallier P, Balland S, Harf R, Valenta R, Deviller P. Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: clinical implications. *Clin Exp Allergy* 1995; **25**:332–9.
- Nieto A, Mazon A, Boquete M *et al*. Assessment of profilin as an allergen for latex-sensitized patients. *Allergy* 2002; **57**:776–84.
- Sussman GL, Beezhold DH, Kurup VP. Allergens and natural rubber proteins. *J Allergy Clin Immunol* 2002; **110**:S33–9.
- Kurup VP, Sussman GL, Yeang HY *et al*. Specific IgE response to purified and recombinant allergens in latex allergy. *Clin Mol Allergy* 2005; **3**:11–20.
- Yip L, Hickey V, Wagner B *et al*. Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000; **121**:292–9.
- Bernstein DI, Biagini RE, Karnani R *et al*. In vivo sensitization to purified *Hevea brasiliensis* proteins in health care workers sensitized to natural rubber latex. *J Allergy Clin Immunol* 2003; **111**:610–6.
- Pamies R, Oliver F, Raulf-Heimsoth M *et al*. Patterns of latex allergen recognition in children sensitized to natural rubber latex. *Pediatr Allergy Immunol* 2006; **17**:55–9.
- Sanz ML, Garcia-Aviles MC, Tabar AI *et al*. Basophil Activation Test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children. *Pediatr Allergy Immunol* 2006; **17**:148–56.
- Mari A, Scala E, D'Ambrosio C, Breiteneder H, Wagner S. Latex allergy within a cohort of not-at-risk subjects with respiratory symptoms: prevalence of latex sensitization and assessment of diagnostic tools. *Int Arch Allergy Immunol* 2007; **143**:135–43.
- Palosuo T, Lehto M, Kotovuori A *et al*. Latex allergy: low prevalence of immunoglobulin E to highly purified proteins Hev b 2 and Hev b 13. *Clin Exp Allergy* 2007; **37**:1502–11.
- Blanco C. Latex-fruit syndrome. *Curr Allergy Asthma Rep* 2003; **3**:47–53.

- 33 Fuchs T, Spitzauer S, Vente C *et al.* Natural latex, grass pollen, and weed pollen share IgE epitopes. *J Allergy Clin Immunol* 1997; **100**:356–64.
- 34 Ganglberger E, Radauer C, Wagner S *et al.* Hev b 8, the Hevea brasiliensis latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. *Int Arch Allergy Immunol* 2001; **125**:216–27.
- 35 Jappe U, Hoffmann M, Hübsch-Müller C, Enk A, Raulf-Heimsoth M. Cross-reactive carbohydrate determinants and in vitro allergy diagnosis: comparison of three conceptionally different automated allergen-specific IgE detection systems. Abstract Book Collegium Internationale Allergologicum, 66. 2008.
- 36 Kolarich D, Altmann F. N-Glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated nonmammalian proteins: application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal Biochem* 2000; **285**:64–75.