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Original Article

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What is already known about this topic? NOVEOS is a new method for specific IgE measurement. Two previous studies described the correlation between NOVEOS and ImmunoCAP, but only for airborne allergens, and did not include clinical data.

What does this article add to our knowledge? Results of ImmunoCAP and NOVEOS specific IgE to 10 food allergens tested in 289 patients were highly correlated. NOVEOS and ImmunoCAP results are both equally predictive of the diagnosis of food allergy.

How does this study impact current management guidelines? For 10 food allergens, this report delivers optimal clinical cutoff values for specific IgE in a mostly pediatric population. Moreover, we addressed the method-related impact of allergen glycosylation on specific IgE results.

BACKGROUND: The clinical significance of newly available platforms for specific IgE measurement must be evaluated. However, data are lacking for NOVEOS (Hycor), especially for food allergens.

OBJECTIVE: We compared the technical and clinical performance of two platforms (ImmunoCAP and NOVEOS) to measure specific IgE to 10 food allergens.

METHODS: Sera from 289 clinically characterized patients were tested for IgE specific for six food allergen extracts (egg white, cow's milk, peanut, hazelnut, fish, and shrimp) and four molecular allergens (Gal d 1, Bos d 8, Ara h 2, and Cor a 14). Specific IgE measurements were carried out using ImmunoCAP and NOVEOS methods. Food allergy diagnoses were established according to international guidelines.

RESULTS: A strong correlation ($\rho > 0.9$) was present between the two platforms whereas specific IgE concentrations measured with NOVEOS were consistently lower (mean, −15%) than with ImmunoCAP. NOVEOS and ImmunoCAP provided similar overall odds ratios and relative risks for food allergy diagnosis with both allergen extracts and molecular allergens. When all 10 allergens were considered, NOVEOS provided better receiver operating characteristic curves ($P = .04$). Finally, we found that the most discordant results were observed with hazelnut and peanut extracts and were related to cross-reactive carbohydrate determinants for these two with ImmunoCAP.

CONCLUSIONS: Specific IgE determination by either ImmunoCAP or NOVEOS (odds ratios of allergy, 25.1 or 33.0, respectively) is highly informative regarding the risk of allergy in the selected population. The NOVEOS platform presents the advantage of being less affected by unwanted reactivity owing to carbohydrate determinant-specific IgE while requiring a 10-fold lower test sample volume.

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Abbreviations used
CCD- Cross-reactive carbohydrate determinant
OFC- Oral food challenge
OIT- Oral immunotherapy
PPV- Positive predictive value
ROC- Receiver operating characteristic curve
RR- Relative risk

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Key words: *Food allergy; Specific IgE platforms; Allergen extract; Molecular allergen; Cross-reactive carbohydrate determinant; Clinical performance*

INTRODUCTION

Determination of specific IgE (sIgE) is one of the pillars supporting allergy diagnosis, together with anamnesis, skin tests, and allergen challenges.[1,2] IgE sensitization is commonly demonstrated *in vivo* by skin prick test, or *in vitro* with automated systems. Because IgE concentrations are low in peripheral blood,[3] sensitive methods for sIgE measurement have been developed, such as ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), which has been used for more than 30 years and is currently considered the reference method.[4,5] Accurate determination of sIgE concentrations is hampered by factors such as the variable composition of the allergenic sources,[6] diverse physicochemical methods used to prepare allergen extracts, possible competition by non-IgE anti-allergen antibodies,[7,8] unwanted reactivity with clinically irrelevant cross-reactive carbohydrate determinants (CCDs),[9] and a lack of result standardization.[10] Initially developed to quantitate sIgE able to bind to allergenic extracts, which are complex mixtures of proteins, contemporary sIgE assays also measure sIgE to a variety of individual allergenic molecules, called molecular allergens (MAs).[11] In daily practice, the added value of these tests resides in the correct identification of the culprit allergen(s) and the estimation of the risk of severe reactions.[12] This risk is higher when individuals are sensitized against certain MAs (eg, seed-storage proteins of peanut and nuts) and lower when sensitization is against MA associated with food-pollen allergy syndromes (eg, PR-10, profilins).[12-15] A commonly accepted rule is that, isolated from the clinical context, sIgE values cannot discriminate between sensitization and allergy. The capacity of sIgE values to predict the presence or absence of allergy symptoms as a function of the degree of sensitization is thus constrained by interindividual variations and the presence of cofactors (eg, exercise, medication, concomitant infection). Determination of useful sIgE threshold values, in particular for food allergens, was previously attempted in many studies using ImmunoCAP tests and demonstrated a general lack of agreement for these values.[16]

In the past 3 years, contenders such as NOVEOS (Hycor, Garden Grove, Calif) have started to propose new methods of sIgE measurement. NOVEOS uses biotinylated soluble allergens coupled with streptavidin-coated magnetic beads and chemiluminescent signals. Thus it differs from ImmunoCAP, which employs allergens bound to a cellulose matrix and fluorescence signals. NOVEOS also differs from ImmunoCAP by requiring a lower test sample volume of 4 μL, compared with 40 μL.

So far, only two reports have compared the analytical performances of NOVEOS and ImmunoCAP, and only for airborne allergens. The first study compared sIgE results for 21 airborne allergens (nine extracts and 12 MAs) on samples from 368 patients[17] and found a good overall correlation (Spearman's ρ: 0.65-0.96 for extracts; and 0.79-0.98 for MA). The second report compared sIgE reactivity against two mixtures of airborne allergens, ImmunoCAP Phadiatop and NOVEOS SX01, on a cohort of 1,314 pediatric samples. Spearman's correlation between the data sets of both methods was 0.84.[18]

However, a comparison of the clinical performance of the two methods has not yet been conducted and data on food allergens are lacking. The main objective of this study was to compare the performance of NOVEOS and ImmunoCAP technologies in a clinical setting of food allergy (FA).

METHODS

Samples, data collection, and ethics statement

This monocentric, noninterventional, retrospective study was conducted on excess serum samples from 289 patients attending the pediatric or adult pulmonology and allergology departments of the Toulouse Teaching Hospital, France, between 2017 and 2021, for a suspicion of allergy to one or several foods. Blood sampling was part of routine allergy explorations, in agreement with current European Academy of Allergy and Clinical Immunology and World Allergy Organization guidelines.[19,20] Additional experiments were performed only on excess serum. According to French law, patients were informed and retained the right to oppose the use of their anonymized medical data and excess samples for research purposes, but formal consent was not required for this noninterventional study, which was categorized as type 3b noninterventional research (Art. L1121-1 Public Health Code, ethics and General Data Protection Regulation committee-approved collection DC20162804).

Single-blind oral food challenges

Single-blind food challenges were supervised by trained practitioners using recommended threshold cumulative doses.[21] A negative oral food challenge (OFC) was defined by the absence of allergy symptoms after consumption of a cumulative dose of tested food: egg white (>5 g of cooked egg equivalent to 650 mg of protein), cow's milk (>8.5 fl oz/254 mL of raw milk equivalent to 8.3 g of whey protein), peanut (>8.7 g of roasted peanut equivalent to 2.2 g of protein), hazelnut (>8.7 g of roasted hazelnut equivalent to 1.3 g of protein), fish (>50 g equivalent to 12.5 g of protein), or shrimp (>39 g equivalent to 7.5 g of protein).

Food allergy diagnostic procedures

Food allergy diagnosis was established based on single-blind OFC results; when an OFC was not performed, it was based on a documented episode of food anaphylaxis and the demonstration of sensitization to culprit foods. In accordance with international guidelines, OFCs were not undertaken in patients with a risk of severe anaphylaxis or in cases of refusal.[22-24] In patients who were allergic to several foods (n = 48 of 289 patients), allergy workup was conducted separately for each food. Some patients were receiving oral immunotherapy (OIT) for the culprit food at the time of blood sampling (n = 104). A separate analysis was performed for patients receiving peanut OIT.

TABLE I. Demographics and clinical status of patients

Allergen	Patients, n	Median age (interquartile range)	Sex ratio	Before FA tests			After FA tests	
				Strict avoidance*	OIT†	Episodic consumption‡	Patients with oral food challenge§	Allergic‖
Peanut	76	8 (6-11)	1.6	9/76	43/76	24/76	76/76	33/76
Cow's milk	60	7 (2-19)	2.0	17/60	28/60	15/60	31/60	31/60
Egg white	44	3 (2-6)	2.6	8/44	18/44	18/44	30/44	15/44
Hazelnut	53	6 (4-10)	2.3	10/53	11/53	32/53	20/53	19/53
Fish	51	9 (4-13)	1.7	27/51	1/51	23/51	24/51	22/51
Shrimp	53	10 (6-15)	1.0	28/53	3/53	22/53	17/53	24/53

FA, food allergy; *OIT*, oral immunotherapy.
We conducted FA tests (n = 337) in 289 patients; 48 of 289 individuals who presented with two FAs were tested for the corresponding allergens.
*Patients following a strict avoidance diet.
†Patients receiving OIT for the tested food at the time of sampling.
‡Patients not following OIT but consuming the culprit food episodically.
§Patients with an FA diagnosis based on oral food challenge.
‖Patients with a confirmed FA diagnosis.

Specific IgE measurements

Specific IgE measurements were performed with ImmunoCAP Phadia 250 (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) and NOVEOS (Hycor, Garden Grove, Calif) systems, in compliance with ISO 15189 standards. After the initial determination by the ImmunoCAP method, samples were kept frozen at −40°C before testing with NOVEOS. For statistical analyses, sIgE values outside the analyzers' ranges of measurement (ImmunoCAP: 0.10-100 kU_A/L; NOVEOS: 0.17-100 kU_A/L) were adjusted to 0.10 or 0.17 kU_A/L, respectively, for results below these values, or to 100 kU_A/L for results greater than 100 kU_A/L. Some samples were tested after the addition of a CCD-blocker reagent (ProGlyCan MUXF3-human serum albumin, Hämosan, Ilz, Austria) at a final concentration of 20 μg/mL.

Results analysis and statistics

Analytical correlations and general agreement between NOVEOS and ImmunoCAP were calculated by using Spearman's formula, Cohen's κ index, and the percentage of agreement (proportion of both true positive and true negative results). Clinical performance of sIgE values was determined through odds ratios (ORs) (Baptista-Pike's CI) and relative risks (RR) (CIs according to Koopman's asymptotic score) of presenting with allergy, and through the calculation of receiver operating characteristic curves (ROCs), κ index rankings (in which 3 indicates moderate; 4, good; and 5, very good), and percentages of agreement (% agreement: true positives + true negative / total number of tests). We compared indicators of clinical performance using Wilcoxon *t* test. For these analyses, individuals were categorized for each allergen and technique into four groups: true positives (confirmed allergy and sIgE above the cutoff value for the relevant allergen), true negatives (confirmed tolerance and sIgE below the cutoff value for the relevant allergen), false negative (confirmed allergy and sIgE below the cutoff value for the relevant allergen), and false positive (confirmed tolerance and sIgE above the cutoff value). These four groups were used to establish contingency tables (Fisher exact test). All statistical calculations were performed using PRISM 9 (GraphPad Software, San Diego, Calif, www.graphpad.com). Significance was set at $P > .05$. Optimal cutoff sIgE values were calculated using Youden's index.[25]

RESULTS

Patient characteristics

The study population was predominantly male (183 of 289 patients), median age 7 years; 246 were aged 15 years or younger. A total of 48 patients were investigated for two distinct FAs, resulting in a total of 337 FA tests (Table I). Owing to a risk of severe anaphylaxis or refusal, only 198 of 337 FA diagnoses were based on OFC, ranging from a minimum of 17 of 53 for shrimp allergy to all 76 for peanut allergy (Table I). The FA tests were performed on patients after either OIT (104 of 337 tests; range, one of 51 for fish to 43 of 76 for peanut) or a strict avoidance diet (99 of 337 tests), or on patients who reported episodic consumption of the investigated food (134 of 377 tests). Overall, 43% of FA tests concluded the presence of allergy.

Comparison of analytical performance of ImmunoCAP and NOVEOS

Before we analyzed the clinical performance of the two methods, we compared ImmunoCAP and NOVEOS sIgE results at the analytical level. A total of 570 comparisons were made encompassing six allergen extracts and four associated MAs: egg white/ovomucoid (nGal d 1), cow's milk/casein (nBos d 8), peanut/rAra h 2, hazelnut/rCor a 14, fish, and shrimp (Figure 1). We evaluated the correlation between the methods using Spearman's test, which showed high ρ coefficients for both allergen extracts (r = .92) and MA (r = .96) (Figure 1, *A* and *B*). Using a Bland-Altman approach to test agreement between the methods (Figure 1, *C* and *D*), we observed significant divergence ($P < .0001$) between absolute differences only for values between 10 and 100 kU_A/L but not between residuals (difference/average). When considering values between 0.1 and 100 kU_A/L, NOVEOS sIgE results were lower than ImmunoCAP results by a mean of −15%, from −13% (extracts; $P < .0001$) to −17% (MAs; $P = .0006$).

Then, we found a good level of agreement (κ index = 0.84; agreement = 0.92) between the methods (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Highest or lowest levels of concordance were found for egg white extract (κ = 1.0; agreement = 1.00) and for shrimp (κ = 0.74) and hazelnut extracts (agreement = 0.81), respectively. To gain further insight into the analysis of discordances, we subdivided

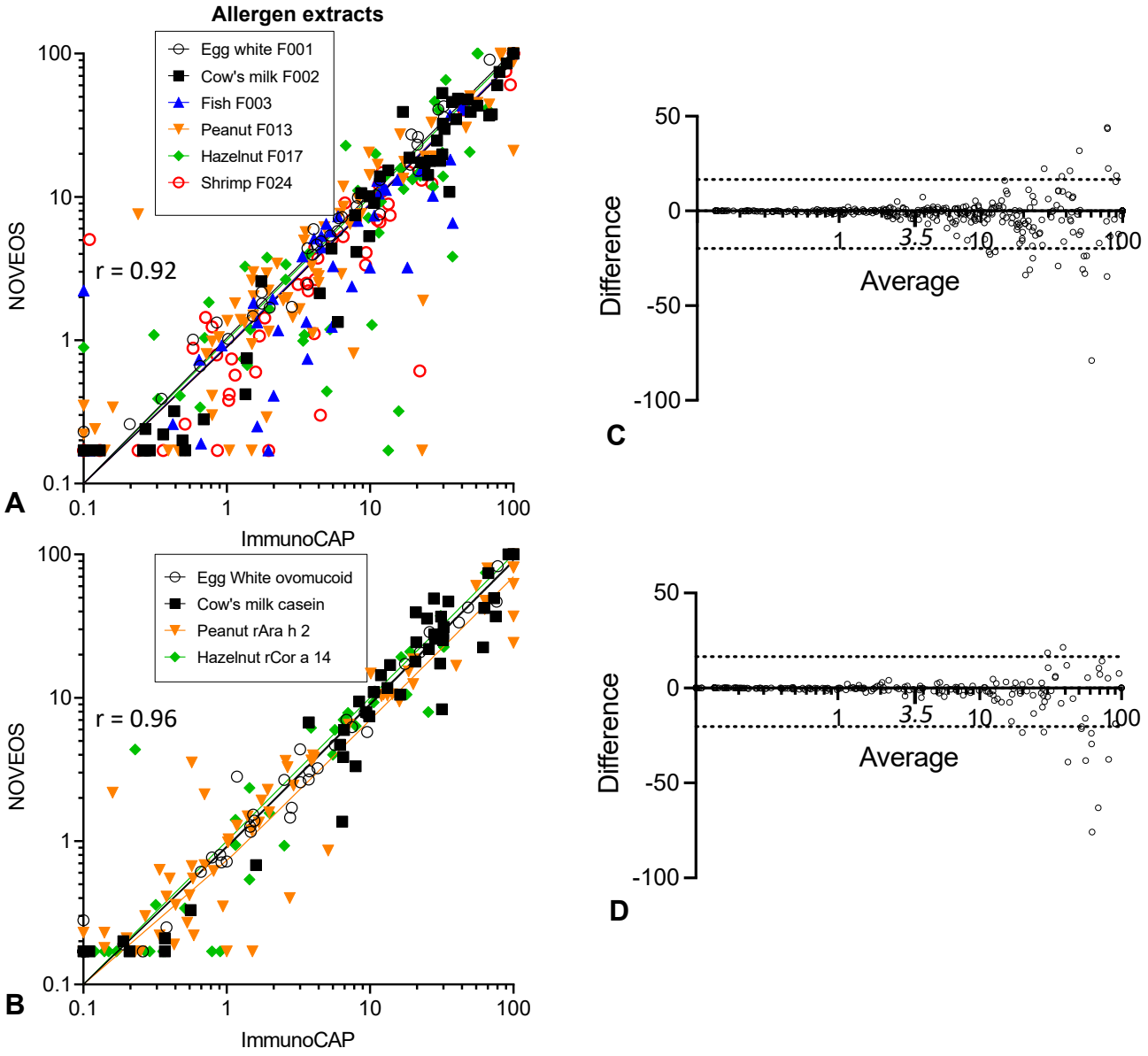

FIGURE 1. Quantitative correlation between ImmunoCAP and NOVEOS. **A**, **C** Allergen extracts (337 data points); **B**, **D** molecular allergens (233 data points). In **C** and **D**, Bland-Altman diagrams plot differences (ordinate values, in kU_A/L) in specific IgE measurements between NOVEOS and ImmunoCAP against average values (abscissa). Ninety-six results exceeded the methods' Limits of detection (72 below 0.1/0.17 and 24 above $100kU_A/L$) and were replaced by their respective limit of detection values. r = Spearman's ρ.

sIgE values according to sIgE reactivity classes from class 0 (≤0.35 kU_A/L) to classes 5 and 6 (>50 kU_A/L) (Figure 2, *A*). These classes allow sIgE analysis to show discrete rather than continuous values.[1] The percentage of agreement within classes was 72% (n = 410 of 570 sIgE results), whereas 23% (133 of 570) results differed by one class. Only 4.7% (27 of 570 results) differed by three or four IgE reactivity classes and corresponded to 25 patients (two patients were discordant for both peanut extract and Ara h 2). These discordant results were mainly for allergen extracts (20 of 27 results), mean sIgE values of 5.3 kU_A/L (range, 0.1-23.4 kU_A/L) for ImmunoCAP and 1.3 kU_A/L (range, 0.13-7.6 kU_A/L) for NOVEOS. For low sIgE values, there was an 86% inter-method agreement for discrimination between sIgE classes 0 (<0.35 kU_A/L) and 1/2 (0.35 to 3.5kU_A/L) (Figure 2, *A*).

Comparison of ImmunoCAP and NOVEOS clinical performance

We performed ROC analysis to compare the clinical performance of ImmunoCAP and NOVEOS and selected sIgE optimal thresholds for discrimination between food-allergic and nonallergic individuals. As presented in Table II and Figure E2 (in this article's Online Repository at www.jaci-inpractice.org), areas under ROCs (AUC) varied from 0.79 for hazelnut extract (ImmunoCAP f17) to 0.97 for ovomucoid nGal d 1 (NOVEOS F233). Mean AUC was higher for NOVEOS than for

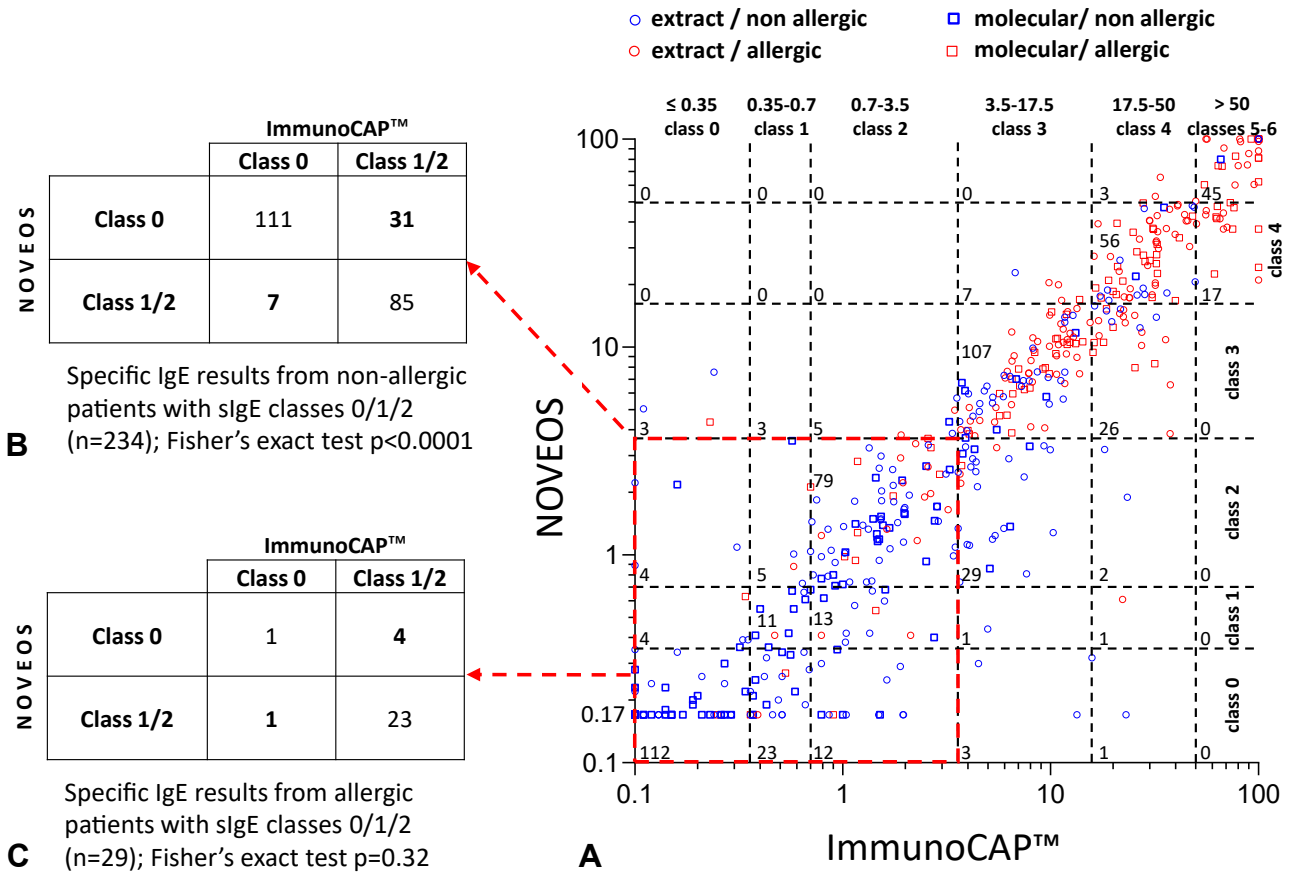

FIGURE 2. Semiquantitative correlation between ImmunoCAP and NOVEOS. **A** Specific IgE results from both methods were grouped into IgE reactivity classes. The number of data points is indicated for all combinations of NOVEOS and ImmunoCAP results. *Red*, patients with allergy; *blue*, patients without allergy; *circles*, extracts; *squares*, molecular allergens. **B**, **C** Specific IgE (sIgE) results (only classes 0, 1, or 2) contingency tables and Fisher exact tests for **B** patients without allergy or **C** patients with allergy.

ImmunoCAP ($P = .04$). Next, we used Youden's index combining optimal sensitivity and specificity to set optimal sIgE cutoff values for the 10 food allergens (Table II). Cutoff values for ImmunoCAP (mean, 6.6 ± 3.9 kU_A/L) and NOVEOS (mean, 4.4 ± 1.9 kU_A/L) were similar (paired samples Wilcoxon test, $P = .07$). The most divergent cutoff values were observed with hazelnut extracts (ImmunoCAP cutoff, 16.7 kU_A/L vs NOVEOS cutoff, 3.6 kU_A/L) and cow's milk extracts (ImmunoCAP cutoff, 6.9 kU_A/L vs NOVEOS cutoff, 3.3 kU_A/L). Sensitivity, specificity, positive predictive values (PPV), or negative predictive values were also comparable without significant differences (mean sensitivity and specificity: 81% and 84% for ImmunoCAP and 84% and 86% for NOVEOS). The highest PPV was 95%, except for egg white extract (ImmunoCAP and NOVEOS: highest PPV = 90%) (Table II). Because some patients were under OIT at the time of sampling, we analyzed the 43 patients receiving peanut OIT separately (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). When we considered only those patients, ROC curves had identical AUC values with both methods: 0.79 (peanut extract) and 0.84 (Ara h 2) (Table E1). We then evaluated the ability of sIgE cutoffs to discriminate allergic from nonallergic individuals by calculating the ORs, RRs, Cohen's κ coefficients, and percent agreements between methods and patients' status. We found a strong association between sIgE results for the 10 allergens and clinical status (Figure 3). Overall values of RRs (ImmunoCAP: 4.1 vs NOVEOS: 4.6), ORs, κ, and agreement were higher for NOVEOS than for ImmunoCAP when data from all 10 allergens were pooled, as well as when only allergen extracts were considered (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). However, these differences were not significant. The ORs and RRs were higher for MAs with ImmunoCAP than with NOVEOS, whereas agreement and κ indexes were identical with both methods (Table E2). In both methods, MAs were associated with better ORs, RRs, agreement and κ indexes than allergen extracts (Table E2). Considering individual allergens (see Table E3 in this article's Online Repository at www.jaci-inpractice.org), cow's milk sIgE results (extract and casein nBos d8) were associated with the highest RRs (8.2), ORs (>100), percent agreement (90% to 92%) and κ indexes (0.80-0.83). The lowest values were obtained with ImmunoCAP peanut extract (RR: 2.7; κ: 0.49; agreement: 75%) and NOVEOS hazelnut extract (RR: 2.6).

Cohen's κ index showed a good (0.61-0.80) or very good (0.81-1) degree of association for six of 10 allergens tested for ImmunoCAP, compared with eight of 10 for NOVEOS (Table E3) ($P = .035$; Wilcoxon t test). In addition, agreement

TABLE II. Summary of ImmunoCAP and NOVEOS clinical performance

Allergen, code*	Type	Method	Area under the curve	Optimal cutoff sIgE level, kU_A/L	Sensitivity	Specificity	Positive predictive value	Negative predictive value	sIgE level for ≥95% positive predictive value, kU_A/L
Peanut, f13	E	I	0.81	5.5	73%	77%	70%	79%	37.8
Peanut, F013	E	N	0.85	7.6	73%	91%	85%	81%	19.8
Peanut rAra h 2, f423	M	I	0.87	2.3	76%	91%	86%	83%	6.1
Peanut rAra h 2, F423	M	N	0.89	1.8	82%	88%	84%	87%	3.7
Egg white, f1	E	I	0.89	6.0	93%	72%	63%	95%	25.8†
Egg white, F001	E	N	0.90	6.5	93%	72%	63%	95%	26.7†
Egg white nGal d 1, f233	M	I	0.95	5.0	87%	93%	87%	93%	10.2
Egg white nGal d 1, F233	M	N	0.97	2.9	100%	83%	75%	100%	7.4
Cow's milk, f2	E	I	0.94	6.9	97%	83%	86%	96%	49.2
Cow's milk, F002	E	N	0.94	3.3	100%	83%	86%	100%	48.2
Casein nBos d 8, f78	M	I	0.95	6.4	97%	86%	88%	96%	48.2
Casein nBos d 8, F078	M	N	0.94	4.3	97%	86%	88%	96%	48.2
Hazelnut, f17	E	I	0.79	16.7	65%	86%	72%	80%	52.8
Hazelnut, F017	E	N	0.82	3.6	90%	70%	64%	92%	56.3
Hazelnut rCor a 14, f439	M	I	0.87	5.6	65%	100%	100%	83%	5.6
Hazelnut rCor a 14, F439	M	N	0.87	4.2	70%	97%	93%	85%	6.2
Fish, f3	E	I	0.87	7.7	72%	90%	84%	81%	37.0
Fish, F003	E	N	0.88	6.5	73%	93%	89%	82%	28.0
Shrimp, f24	E	I	0.85	3.6	92%	69%	71%	91%	40.0
Shrimp, F024	E	N	0.86	3.5	83%	79%	77%	85%	16.0

E, allergen extract; *I*, ImmunoCAP (Thermo Fisher); *M*, molecular; *N*, NOVEOS (Hycor); *sIgE*, specific IgE.
Cutoff values (sIgE kU_A/L) correspond to optimal correlations between sIgE values and food allergy diagnosis (highest Youden index). sIgE concentrations associated with positive predictive value > 95% are in kU_A/L.
*Allergen source and identification.
†Maximum positive predictive value = 90%.

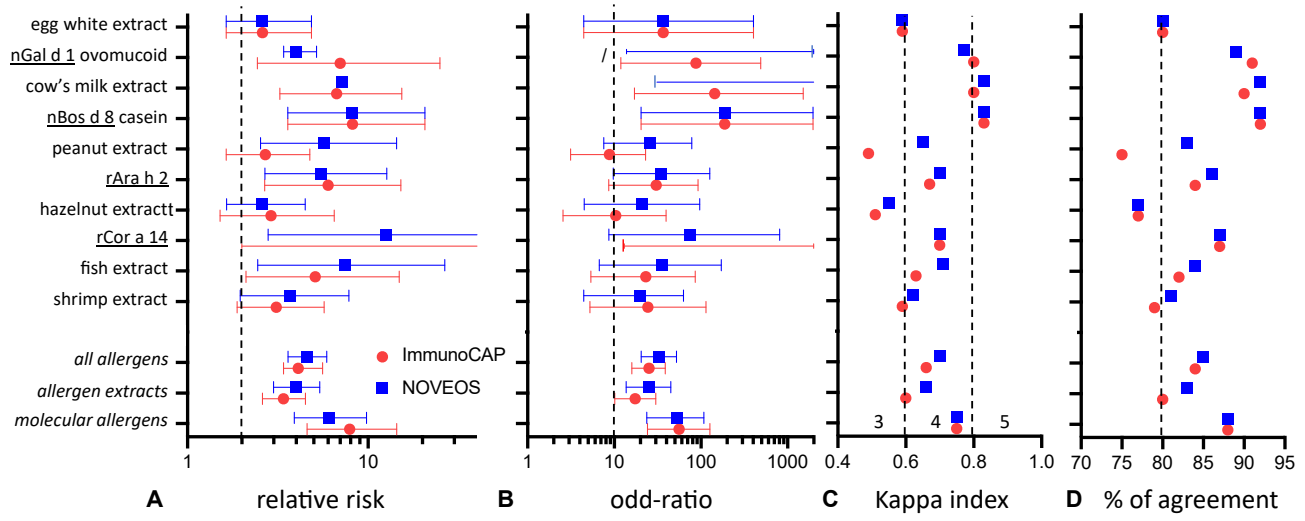

FIGURE 3. **A** Relative risk, **B** odds ratio (OR), **C** κ index, and **D** percent agreement of clinical allergy assessed from specific IgE levels. Individuals were categorized according to their food allergy or food tolerance and to the specific IgE cutoff for each allergen. The OR and relative risk of FA were calculated from contingency tables. *Red circles*, ImmunoCAP; *blue squares*, NOVEOS; *underline*, molecular allergens; *dashed lines*, thresholds for clinical pertinence: relative risk > 2, OR > 10, κ index rankings for degree of agreement with clinical status and percent agreement >80%.

was higher than 80% for nine of 10 allergens in NOVEOS and for seven of 10 allergens in ImmunoCAP (Table E3). Overall, in a comparison of κ indexes and agreement for 10 allergens (ie, 20 comparisons, Wilcoxon *t* test: $P < .0001$), values were significantly higher for NOVEOS (11 of 20) than for ImmunoCAP (two of 20).

TABLE III. MUXF3-positive samples show reactivity against peanut or hazelnut extracts with ImmunoCAP f13/f17 reagents but not with NOVEOS F013/F017

					ImmunoCAP		NOVEOS
Sample no.	Age, y	Sex	MUXF3*	Allergen extract	Without CCD blocker	With CCD-blocker†	Without CCD blocker
1	8	M	2.98	Peanut	23.10	<0.1	<0.1
2	13	F	8.43	Hazelnut	13.4	0.13	0.17
3	9	M	1.81	Hazelnut	3.27	0.22	0.22
4	67	M	1.15	Hazelnut	0.46	<0.1	<0.1
5	71	M	2.19	Hazelnut	0.60	<0.1	<0.1
6	70	F	1.63	Hazelnut	3.96	<0.1	<0.1
7	46	M	15.7	Hazelnut	4.87	0.80	<0.1
8	78	M	8.30	Hazelnut	20.0	<0.1	0.80
9	34	M	3.50	Hazelnut	1.95	<0.1	<0.1
10	32	M	9.29	Hazelnut	14.6	0.14	0.15
11	26	M	44.0	Hazelnut	7.04	0.17	<0.1

CCD, cross-reactive carbohydrate determinant.
Samples 1-3 were chosen for discordance for peanut or hazelnut extract: positive with ImmunoCAP vs negative/very low for NOVEOS. Patients 4-11 are not from the main study and were selected for MUXF3-positivity during routine testing for sensitization against Hymenoptera venom. All values are in kU_A/L.
*MUXF3 specific IgE values were measured using ImmunoCAP o214.
†CCD blocker reagent (MUXF3-HSA) was added.

Focusing on low sIgE values, we performed contingency analyses between class 0 ($\leq$0.35 kU_A/L) and class 1 or 2 (0.35 to 3.5 kU_A/L) measurements, first for nonallergic patients (n = 234) (Figure 2, *B*) and then for allergic patients (n = 29) (Figure 2, *C*). The distribution of sIgE results for nonallergic patients differed between methods, with an excess of 31 ImmunoCAP class 1 or 2 results paired with class 0 NOVEOS results ($P < .0001$). By contrast, no significant difference was found for allergic patients with class 0 to 2 sIgE (Figure 2, *C*).

Origin of discordances between ImmunoCAP and NOVEOS

The 27 most divergent sIgE results from Figure 2, *A* are shown in detail in Figure E3 (in this article's Online Repository at www.jaci-inpractice.org). Because most discordant results concerned plant allergens (18 of 27), we investigated two possible causes. The first potential explanation is the spiking of ImmunoCAP but not NOVEOS hazelnut extract with Cor a 1, a member of the PR-10 MA family.[26] Thus, we assayed the 53 hazelnut-sensitized samples for anti-Cor a 1 sIgE using the Cor a 1 NOVEOS reagent F428. Sera with high concentrations of anti-Cor a 1 sIgE (>10 kU_A/L; range, 11 to >100 kU_A/L; n = 12) were excluded from new ROC calculations. The new AUCs were 0.81 for ImmunoCAP and 0.84 for NOVEOS (data not shown), compared with previous values of 0.79 for ImmunoCAP and 0.82 for NOVEOS before removal of the anti-Cor a 1 sIgE-positive samples. Cutoff values were unchanged (16.7 for ImmunoCAP vs 3.6 for NOVEOS).

The second potential cause for discordant results resides in CCDs displayed by plant allergens. We hypothesized that peanut- or hazelnut-positive results obtained using ImmunoCAP but not NOVEOS were related to IgE reactivity against CCDs. We were able to test 11 of 25 samples for CCD IgE reactivity using ImmunoCAP MUXF3 (o214) reagent, demonstrating anti-MUXF3 IgE in eight of 11. We then used a reagent blocking CCD antibody reactivity (MUXF3-HSA, ProGlyCan reagent from Hämosan) for the three samples that were most discordant for peanut (one patient) and hazelnut extracts (two patients). The CCD-blocker reagent abolished ImmunoCAP reactivity against peanut and hazelnut extracts for these three samples, whereas testing these same samples with NOVEOS revealed no reactivity to peanut or hazelnut extracts without addition of the CCD blocker (Table III). To confirm these results in a different setting, we selected MUXF3-positive samples from eight adult patients with *Hymenoptera* venom sensitization but no history of FA. All of these samples showed sIgE reactivity against ImmunoCAP hazelnut extract but no or low reactivity against NOVEOS hazelnut extract. ImmunoCAP reactivity was abolished or strongly reduced after the addition of the CCD blocker reagent (Table III).

DISCUSSION

We report the compared performance of two sIgE platforms and their clinical cutoffs for 10 common food allergens. Clinical cutoffs for sIgE have previously been proposed multiple times, in particular for food allergens, as indicators of the probability of presenting with allergic symptoms, rather than thresholds accurately predicting the occurrence of symptoms.[15,27,28] Thus, the quantitative nature of sIgE measurements is essential for allergy diagnosis, and physicians must be aware of the characteristics of the methods that are employed. Indeed, several routine methods of sIgE quantitation coexist, belonging to successive generations and to different times of availability for clinical use. First-generation tests were radioimmunoassays that used allergens bound to paper disks and an anti-IgE reagent labeled with a radioisotope, usually ^{125}I. The RadioAllergoSorbent Test (Pharmacia Diagnostics AB, Uppsala, Sweden) was commercialized in 1974.[29] The main second-generation test, ImmunoCAP (Pharmacia AB, now Thermo Fisher Scientific), has allergens covalently bound to a nitrocellulose sponge and uses fluorescent signals to quantify anti-IgE.[4] Third-generation sIgE tests are represented by the IMMULITE 2000 system (Siemens Healthcare SAS, Saint-Denis, France), with biotinylated soluble allergens binding to a large (25-mm-diameter) avidin-coated

unique bead, and chemiluminescent signals for anti-IgE binding.[30] In 2020, fourth-generation technologies for sIgE determination became available (NOVEOS, Hycor; and IDS-iSYS, Bolton, United Kingdom), differing from third-generation tests through the use of biotin-labeled allergens bound to avidin microbeads.[17,31]

Here, we found that NOVEOS sIgE results were significantly lower than those obtained with ImmunoCAP, by a mean value of 15%. This discrepancy does not result from a defect in the linearity of NOVEOS technology[32] that is similar to that of ImmunoCAP.[31] The differences we observed between methods could be due to an underestimation by NOVEOS or an overestimation by ImmunoCAP. An underestimation by NOVEOS could result from a lower density of allergen molecules on NOVEOS beads compared with ImmunoCAP solid phase. Significant discrepancies were previously reported between ImmunoCAP and Immulite.[33] In our work, we observed significant analytical differences mostly for higher (>3.5-kU_A/L) sIgE concentrations (Figure 1, *C* and *D*). The clinical significance of these discrepancies is probably low, especially for sIgE measurements greater than the clinically relevant cutoffs.

By contrast, in sensitized individuals with low sIgE values (≤0.35 to 3.5 kU_A/L; IgE reactivity classes 0-2), we found a better negative predictive value with NOVEOS (Figure 2, *B* and *C*). Correct quantification of low levels of sIgE is important for early detection of sensitization against food allergens in children.[34,35] These results need to be confirmed by further prospective studies.

We also investigated two potential causes for some false-positive results for peanut and hazelnut extracts in nonallergic patients tested with ImmunoCAP: Cor a 1 spiking of ImmunoCAP hazelnut extract and the presence of CCD on plant allergen extracts. Whereas Cor a 1 spiking of ImmunoCAP hazelnut extract did not contribute to clinical performance discrepancies, CCD did. Thus, our study supports the view that glycosylated epitopes are more accessible to sIgE with ImmunoCAP than with NOVEOS. This could be due to the avidin-coated beads and the biotinylation of NOVEOS allergens. Another possibility is that anti-CCD sIgE react with CCD determinants on allergen molecules and also with the nitrocellulose sponge matrix on ImmunoCAP.[36,37] Unlike the animal-derived galactose-α-1,3-galactose epitope, plant CCDs (eg, MUXF3), are currently considered to be devoid of clinical relevance in allergy.[38] This could explain the better correlation between plant FA and NOVEOS sIgE results. As expected, recombinant, nonglycosylated MA displayed similar clinical performance.

Despite these discrepancies, we report that NOVEOS and ImmunoCAP have mostly similar performance in discriminating between food-allergic and nonallergic individuals. It is impossible to determine clinically relevant universal thresholds of sIgE concentrations because of important variations from one population to another. For example, the proposed cutoff for rAra h 2 sIgE concentration in peanut allergy varies from 0.10 to 42.2 kU_A/L.[16] However, the establishment of local clinical cutoffs is of utmost importance for managing a given population, including the design of OFC protocols. In support of this assertion, a 2002 study conducted in our center found a clinical threshold for ImmunoCAP peanut extract sIgE of 15kU_A/L with 95% specificity and 44% sensitivity, which was similar to values reported in this 15- to 20-year later study (cutoff of 14kU_A/L; 86% specificity and 51% sensitivity).[28] Thus, cutoff values can be established for a given population on the condition of using similar protocols, and appear to be stable over time. Our study further supports that sIgE measurements by either ImmunoCAP or NOVEOS are highly informative regarding the risk of allergy, with an OR greater than 10 and RR greater than 2 in the population in this work.

There are several limitations to our study. It has a retrospective and monocentric design, its population was predominantly pediatric (85%), OFC was not systematically included in the FA workup except for peanut, and observance of a strict avoidance diet, from 12% (peanut) to 53% (seafood), was variable. Moreover, we performed single-blind rather than double-blind OFCs; the latter is considered the reference standard for diagnosing FA. Nevertheless, single-blind OFC is recognized as a valuable diagnosis tool.[22] On the other hand, to our knowledge, our study is the first to provide extensive intermethod comparison addressing both extracts and molecular food allergens. Moreover, the heterogeneity of patients and their therapeutic protocols mirrors real-life practice in our center.

We demonstrate here that for 10 common food allergen extracts and molecules assayed in a large cohort, sIgE determination performed with either NOVEOS or ImmunoCAP is highly correlated with and predictive of the actual diagnosis of FA or tolerance. However, NOVEOS requires a 10-fold lower test sample volume (4 μL) compared with ImmunoCAP (40 μL), which can be advantageous for allergy diagnosis in children. Further confirmatory studies are warranted, including more allergens (ie, other food allergens, respiratory, venom, drugs) and both adult and pediatric patients from other geographic areas. However, allergology practitioners must remember that regardless of the method used to measure sIgE, these methods are tools aimed at improving the physician's clinical analysis and decision but are insufficient in themselves to establish an allergy diagnosis.

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TABLE E1. Performance of ImmunoCAP and NOVEOS in 43 patients receiving peanut oral immunotherapy

Allergen, code	Type	Method	Receiver operating characteristic curve AUC	Cutoff	Sensitivity	Specificity	Positive predictive value	Negative predictive value	≥95% positive predictive value
Peanut, f13	E	I	0.79	9.2	56%	94%	93%	61%	20.7
Peanut, F013	E	N	0.79	7.6	72%	89%	90%	70%	25.9
Peanut rAra h 2, f423	M	I	0.84	2.3	72%	94%	95%	71%	6.1
Peanut rAra h 2, F423	M	N	0.84	2.3	72%	89%	90%	70%	3.6

AUC, area under curve; *E*, allergen extract; *I*, ImmunoCAP (Thermo Fisher); *M*, molecular; *N*, NOVEOS (Hycor).
Receiver operating characteristic curves and clinical performance parameters were calculated for 43 patients (out of a total of 77 studied for peanut sIgE) receiving peanut oral immunotherapy at the time of the study. Cutoff values and specific IgE concentrations associated with >95% positive predictive values are in kU_A/L.

TABLE E2. Odds ratios and relative risks of clinical allergy assessed from specific IgE concentrations

Method	Allergens	Odds ratio (95% CI)	Relative ratio (95% CI)	% Agreement	κ index
ImmunoCAP	All	25.1 (15.9-38.8)	4.1 (3.4-5.6)	84%	0.66
NOVEOS	All	33.0 (20.5-52.1)	4.6 (3.6-5.9)	85%	0.70
ImmunoCAP	Extracts	17.4 (10.1-30.1)	3.4 (2.6-4.5	80%	0.60
NOVEOS	Extracts	25.1 (13.7-44.8)	4.0 (3.0-5.4)	83%	0.66
ImmunoCAP	Molecular	55.8 (24.2-127)	7.9 (4.6-14.4)	88%	0.75
NOVEOS	Molecular	52.5 (23.7-108)	6.1 (3.9-9.8)	88%	0.75

Contingency tables were established by regrouping results according to clinical status and allergen cutoffs. Odds ratio (Baptista-Pike), relative ratio (Koopman), and κ index (Cohen) were calculated separately for ImmunoCAP and NOVEOS methods and for allergens extracts (f1, f2, f3, f13, f17, and f24), molecular allergens (f233, f78, f423, and f439) or for all allergens. Percent agreement between specific IgE dosages and clinical status was calculated as: (true positives + true negatives) / total number of patients.

TABLE E3. Contingency tables and analysis (Fisher exact test) to evaluate ORs (Baptista-Pike) and RRs (Koopman) of presenting with clinical allergy depending on specific IgE levels

ImmunoCAP					NOVEOS				
Allergen cutoff	**Nonallergic**	**Allergic**	**% Agreement**	**κ index**	**Allergen cutoff**	**Nonallergic**	**Allergic**	**% Agreement**	**κ index**
Egg white					Egg white				
<6 KU_A/L	21	1	80%	0.59	<6.5 KU_A/L	21	1	80%	0.59
>6 KU_A/L	8	14			>6.5 KU_A/L	8	14		
$P < .0001$, OR = 36.7, RR = 2.6					$P < .0001$, OR = 36.7, RR = 2.6				
Ovomucoid nGal d 1					Ovomucoid nGal d 1				
<5 KU_A/L	27	2	91%	0.80	<2.9 KU_A/L	24	0	89%	0.77
>5 KU_A/L	2	13			>2.9 KU_A/L	5	15		
$P < .0001$, OR = 87.7, RR = 7					$P < .0001$, OR = NC, RR = 4.0				
Cow's milk					Cow's milk				
<6.9 KU_A/L	24	1	90%	0.80	<3.3 KU_A/L	24	0	92%	0.83
>6.9 KU_A/L	5	30			>3.3KU_A/L	5	31		
$P < .0001$, OR = 144.0, RR = 6.7					$P < .0001$, OR = NC, RR = 7.2				
Casein nBos d 8					Casein nBos d 8				
<6.4 KU_A/L	25	1	92%	0.83	<4.3 KU_A/L	25	1	92%	0.83
>6.4 KU_A/L	4	30			>4.3 KU_A/L	4	30		
$P < .0001$, OR = 187.5, RR = 8.2					$P < .0001$, OR = 187.5, RR = 8.2				
Peanut					Peanut				
<5.5 KU_A/L	33	9	75%	0.49	<7.6 KU_A/L	39	9	83%	0.65
>5.5 KU_A/L	10	24			>7.6 KU_A/L	4	24		
$P < .0001$, OR = 8.8, RR = 2.7					$P < .0001$, OR = 26.0, RR = 5.7				
Peanut rAra h 2					Peanut rAra h 2				
<2.3 KU_A/L	39	8	84%	0.67	<1.8 KU_A/L	38	6	86%	0.70
>2.3 KU_A/L	4	25			>1.8 KU_A/L	5	27		
$P < .0001$, OR = 30.5, RR = 6.0					$P < .0001$, OR = 34.2, RR = 5.5				
Hazelnut					Hazelnut				
<16.7 KU_A/L	28	7	77%	0.51	<3.6 KU_A/L	23	2	77%	0.55
>16.7 KU_A/L	5	13			>3.6 KU_A/L	10	18		
$P = .0001$, OR = 10.4, RR = 2.9					$P < .0001$, OR = 20.7, RR = 2.6				
Hazelnut rCor a 14					Hazelnut rCor a 14				
<5.6 KU_A/L	33	7	87%	0.70	<4.2 KU_A/L	32	6	87%	0.70
>5.6 KU_A/L	0	13			>4.2 KU_A/L	1	14		
$P < .0001$, OR = NC, RR = NC					$P < .0001$, OR = 74.7, RR = 12.6				
Fish					Fish				
<7.7 KU_A/L	26	6	82%	0.63	<6.5 KU_A/L	27	6	84%	0.71
>7.7 KU_A/L	3	16			>6.5 KU_A/L	2	16		
$P < .0001$, OR = 23.1, RR = 5.1					$P < .0001$, OR = 36.0, RR = 7.4				
Shrimp					Shrimp				
<3.6 KU_A/L	20	2	79%	0.59	<3.5 KU_A/L	23	4	81%	0.62
>3.6 KU_A/L	9	22			>3.5 KU_A/L	6	20		
$P < .0001$, OR = 24.4, RR = 3.1					$P < .0001$, OR = 19.7, RR = 3.7				

NC, not calculable; *OR*, odds ratio; *RR*, relative risk.

For each allergen, optimal cutoff values from Table 1 are indicated on the left and the number of patients above or below that value is shown for the two categories of allergic or nonallergic patients. *P* values are from Fisher exact tests.

	kappa index		*% of agreement*	
	extracts	moleculars	extracts	moleculars
egg's white	**1.00**	0.77 ±0.1	1.00	0.89
cow's milk	**0.97 ±0.03**	**0.86 ±0.07**	0.98	0.93
peanut	**0.84 ±0.06**	**0.81 ±0.07**	0.92	0.91
hazelnut	**0.83 ±0.10**	**0.90 ±0.07**	0.81	0.96
fish	**0.87 ±0.07**		0.94	
shrimp	0.74 ±0.09		0.87	
all extracts	**0.84 ±0.03**		0.92	
all moleculars		**0.84 ±0.04**		0.92

Kappa	
very good	**0.81-1.00**
good	0.61-0.80
moderate	0.41-0.60
fair	0.21-040
poor	0.00-0.20

FIGURE E1. Agreement between NOVEOS and ImmunoCAP results. Cohen's κ index and percent agreement were calculated for each allergen tested. Results were considered to be in agreement when specific IgE results from the two methods were below or above their respective cutoffs listed in Table II.

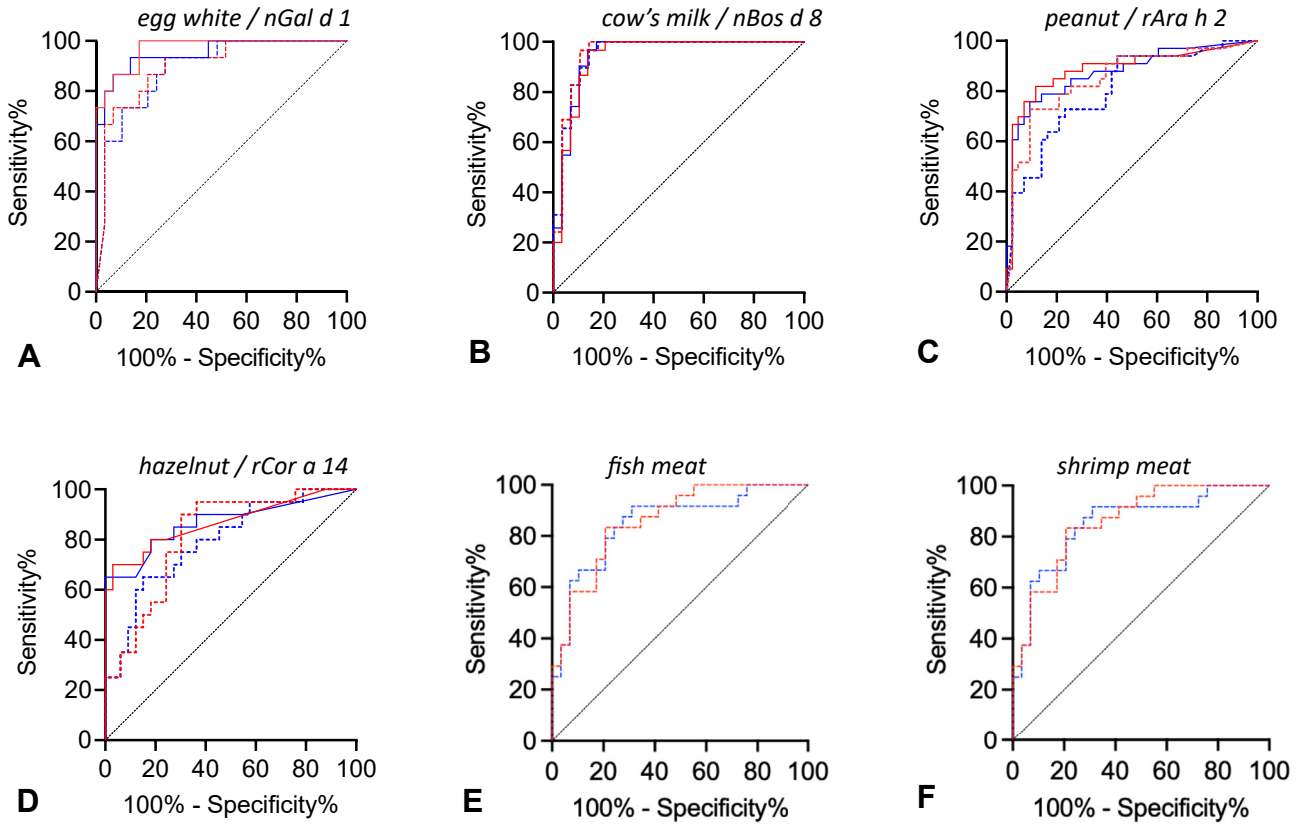

FIGURE E2. Receiver operating characteristic curve (ROC) curves for ImmunoCAP and NOVEOS. *Blue*, ImmunoCAP ROC curves; *red*, NOVEOS ROC curves. **A**Egg white f1 (*dashed lines*), nGal d 1 ovomucoid (*solid lines*); **B** cow's milk extract f2 (*dashed lines*), nBos d 8 casein f78 (*solid lines*); **C** peanut extract f13 (*dashed lines*), rAra h 2 f423 (*solid lines*); **D** hazelnut extract f17 (*dashed lines*), rCor a 14 f439 (*solid lines*); **E** fish extract f3; **F** shrimp extract f24. The areas under the curve of ROC curves are listed in Table II.

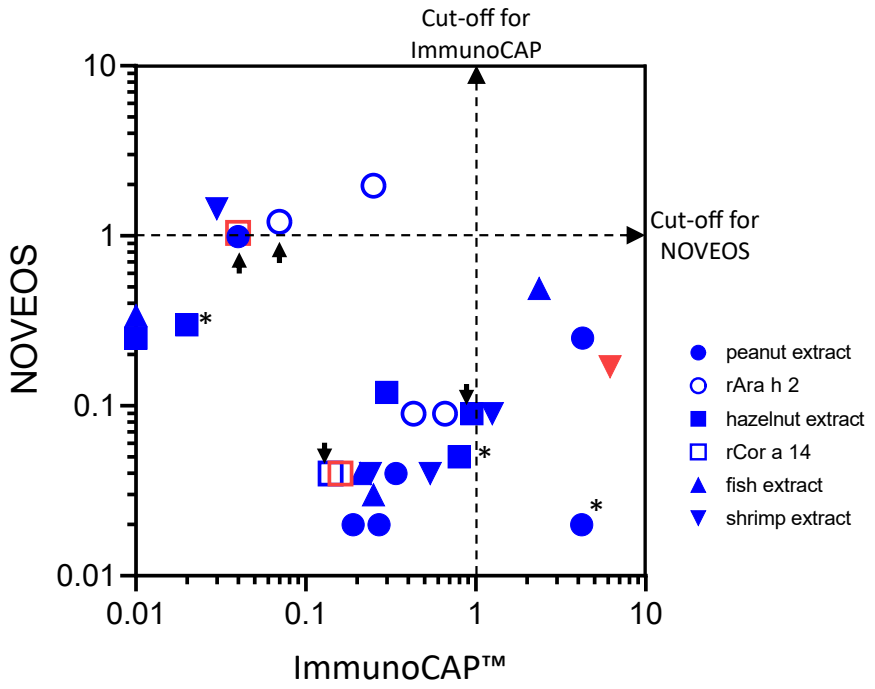

FIGURE E3. Analysis of large discordances between NOVEOS and ImmunoCAP results. ImmunoCAP and NOVEOS specific IgE (sIgE) values that were discordant by at least two IgE reactivity classes are shown (n = 27 results of 570 sIgE measurements). For a given allergen, the sIgE result is shown as the ratio of the sIgE concentration (in kU_A/L) divided by the optimal cutoff for that allergen (as specified in Table II), in which a value of 1 corresponds to the cutoff value. Patients are categorized as allergic (*red symbols*) or nonallergic (*blue symbols*). *Arrows* designate extracts and molecular allergen results from the same two patients. *Asterisks* denote the three patients tested with a cross-reactive carbohydrate determinant inhibitor (patients 1, 2, and 3 in Table III). Discordant sIgE results were for peanut extract (n = 6), rAra h 2 (n = 4), hazelnut extract (n = 5), rCor a 14 (n = 3), fish extracts (n = 4), and shrimp extracts (n = 5). Using the cutoff values established previously, nine patients were incorrectly classified as allergic": four by NOVEOS and five by ImmunoCAP. Two allergic patients were correctly categorized by one method but not by the other: one patient allergic to shrimp was above the ImmunoCAP cutoff for this allergen but below the NOVEOS cutoff, and one patient allergic to hazelnut was above the NOVEOS cutoff (for both hazelnut extract and rCor a 14) but below the ImmunoCAP cutoff. Finally, one patient who was allergic to hazelnut was misclassified (below clinical cutoffs) by both methods and both hazelnut allergens (rCor a 14 and extract).