

# Journal Pre-proof

NOVEOS™ and ImmunoCAP™ have similar performances for diagnosing food allergies.

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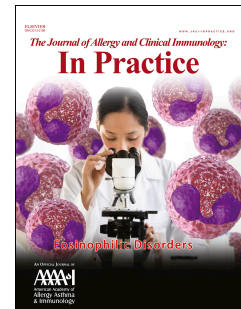
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**TITLE:**

NOVEOS™ and ImmunoCAP™ have similar performances for diagnosing food allergies.

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**Abstract**

**Background:** The clinical interest of newly available platforms for specific IgE measurement must be evaluated. However, data are lacking for NOVEOS™ (Hycor), in particular for food allergens.

**Objective:** We compared technical and clinical performance of two platforms (ImmunoCAP™ and NOVEOS™) for the measurement of 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, shrimp) and four molecular allergens (Gal d 1, Bos d 8, Ara h 2, Cor a 14). Specific IgE measurements were carried out using ImmunoCAP™ and NOVEOS™ methods. Food allergy diagnosis was established according to international guidelines.

**Results:** A strong correlation ( $\rho > 0.9$ ) was present between the two platforms, while 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 ROC curves ( $p = 0.04$ ). Finally, we found that the most discordant results were observed with hazelnut and peanut extracts, and were related to cross-reactive carbohydrate determinants on these two ImmunoCAP™.

**Conclusions:** Specific IgE determination by either ImmunoCAP™ or NOVEOS™ (odd-ratios of allergy were 25.1 or 33.0, respectively) is highly informative on the risk of allergy in the selected population. The NOVEOS™ platform presents the advantage of being less affected by unwanted reactivity due to carbohydrate determinants-specific IgE, while requiring a ten-fold lower test sample volume.

#### Highlights box

1. 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.
2. 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.
3. How does this study impact current management guidelines? For 10 food allergens, this report delivers optimal clinical cut-off values for specific IgE in a mostly pediatric population. Moreover, we addressed the method-related impact of allergen glycosylation on specific IgE results.

#### Keywords:

food allergy, specific IgE platforms, allergen extract, molecular allergen, cross-reactive carbohydrate determinant, clinical performance

**Author contribution:**

The study was designed by PAA and BTE. Data were acquired by BTE, ADLC and PAA with technical assistance from ADLC and CT. AMB, MM, VG, AD, LG and CM are clinical allergologists and provided anonymous patients' data. BTE and PAA wrote the article which was reviewed by JV, JG and CK. All authors approved the final version of the article.

**Conflicts of interest:**

JV reports speaker and consultancy fees in the past 5 years from Astra Zeneca, HpVac, L'Oréal, Meda Pharma (Mylan), Novartis, Sanofi, Thermo Fisher Scientific, outside the submitted work. The other authors declare having no competing interests in relation to this study.

**Abbreviations:**

CCD, cross-reactive carbohydrates; C.I., confidence interval; Eq, equivalent; Ig, immunoglobulin; NPV, negative predictive value; OFC, oral food challenge; OIT, oral immunotherapy; OR, odds ratio; oz, ounce; PEG, polyethylene-glycol; PPV, positive predictive value; ROC, receiver-operator curve; RR, relative risk; SPT, skin prick test; y.o. years old.

## 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 testing (SPT), or *in vitro* with automated systems. Because IgE concentrations are very low in peripheral blood (3), very sensitive methods for sIgE measurement have been developed, such as ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden), which has been in use for more than 30 years and is currently considered as the reference method (4, 5). Accurate determination of sIgE concentrations is hampered by factors such as variable composition of the allergenic sources (6), diverse physico-chemical 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 (CCD) (9), and lack of result standardization (10). Initially developed to quantitate sIgE capable of binding 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 (MA) (11). In daily practice, the added value of these tests resides in the correct identification of the culprit allergen(s) and in the estimation of the risk of severe reactions (12). This risk is higher when individuals are sensitized against certain MA, (e.g. seed-storage proteins of peanut and nuts), and lower when sensitization is against MA associated with food-pollen allergy syndromes (e.g. 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 co-factors (e.g. exercise, medication, concomitant infection, etc). 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 last three years, new contenders, like NOVEOS™ (Hycor, Garden Grove, CA, USA), have started to propose new methods of sIgE measurement. NOVEOS™ uses biotinylated soluble allergens coupled to streptavidin-coated magnetic beads and chemiluminescent signals, thus differs from ImmunoCAP™, which employs allergens bound to a cellulose matrix and fluorescence signals. NOVEOS™ differs also from ImmunoCAP™ by requiring a lower test sample volume of 4 µL, versus 40 µL.

So far, only two reports have compared analytical performances of NOVEOS™ and ImmunoCAP™, and only for airborne allergens. The first study compared sIgE results for 21 airborne allergens (9 extracts and 12 MA) on samples from 368 patients (17) and found a good overall correlation (Spearman’s rho: 0.65-0.96 for extracts; 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 1314 pediatric samples. Spearman’s correlation between the data set of both methods was 0.84 (18).

However, comparison of the clinical performance of the two methods has not been addressed yet, 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).

## Materials and methods

### *Samples, data collection and ethics statement*

This monocentric, non-interventional, retrospective study was conducted on excess serum samples from 289 patients attending the paediatric 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 EAACI and WAO guidelines (19, 20). Additional experiments were performed only on excess serum. According to French law, the 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 non-interventional study categorized as type 3b non-interventional research (Art. L1121-1 Public Health Code, ethics and GDPR committee approved collection DC20162804).

#### *Single-blind Oral food challenges (OFC)*

Single-blind food challenges were supervised by trained practitioners using recommended threshold cumulative doses (21). A negative 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 Eq to 650 mg of protein), cow's milk (>8.5 fl oz/254 mL of raw milk Eq to 8.3 g of whey protein), peanut (>8.7 g of roasted peanut Eq to 2.2 g of protein), hazelnut (>8.7 g of roasted hazelnut Eq to 1.3 g of protein), fish (>50 g Eq to 12.5 g of protein) or shrimp (>39 g Eq to 7.5 g of protein).

#### *Food allergy diagnostic procedures*

FA diagnosis was established either based on single-blind oral food challenge (OFC) results and/or, when an OFC was not performed, 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, 23, 24). In patients allergic to several foods (n=48 patients out of 289), allergy work-up 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.

#### *Specific IgE measurements*

Specific IgE measurements were performed with ImmunoCAP™ Phadia 250 (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) and NOVEOS™ (Hycor, Garden Grove, CA, USA) systems, in compliance with ISO 15189 standards. Following 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<sub>A</sub>/L; NOVEOS™: 0.17 - 100 kU<sub>A</sub>/L) were adjusted to 0.10 or 0.17 kU<sub>A</sub>/L respectively for results below these values, or to 100 kU<sub>A</sub>/L for results >100 kU<sub>A</sub>/L. Some samples were tested after 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 the Spearman's formula, Cohen's kappa index ( $\kappa$ ), and percentage of agreement (proportion of both true positive and true negative results). Clinical performance of specific IgE values was determined through odds-ratios (OR; Baptista-Pike's confidence intervals) and relative risks (RR; C.I. according to Koopman's asymptotic score) of presenting with allergy, and also through calculation of receiver operator curves (ROC), kappa index rankings (3: moderate, 4: good, 5: very good) and

percentages of agreement (% of agreement: true positives + true negative / total number of tests). Indicators of clinical performance were compared 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 cut-off value for the relevant allergen), “true negatives” (confirmed tolerance and sIgE below the cut-off value for the relevant allergen), “false negative” (confirmed allergy and sIgE below the cut-off value for the relevant allergen), and “false positive” (confirmed tolerance and sIgE above the cut-off value). These four groups were used to establish contingency tables (Fisher’s exact test). All statistical calculations were performed by using PRISM 9 (GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com)). Significance was set at  $p > 0.05$ . Optimal cut-off sIgE values were calculated using Youden’s index (25).

## Results

### *Patients characteristics*

The study population (289 patients) was predominantly male (183/289), displayed a median age of 7 years, with 246/289 aged 15 years or younger. Forty-eight patients were investigated for two distinct FA, resulting in a total of 337 FA testings (Table 1). Due to a risk of severe anaphylaxis or refusal, only 198/337 FA diagnoses were based on OFC, ranging from a minimum of 17/53 for shrimp allergy to 76/76 for peanut allergy (Table 1). FA testings were performed on patients either following OIT (104/337 testings; range 1/51 for fish to 43/76 for peanut), or following a strict avoidance diet (99/337 testings), or on patients who reported episodic consumption of the investigated food (134/377 testings). Overall, 43% of FA testings concluded to the presence of allergy.

### *Comparison of analytical performance of ImmunoCAP™ and NOVEOS™*

Prior to analysing 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 6 different allergen extracts and 4 associated MA: egg white/ovomuroid (nGal d 1), cow’s milk/casein (nBos d 8), peanut/rAra h 2, hazelnut/rCor a 14, fish and shrimp (Figure 1). The correlation between the two methods was evaluated using the Spearman’s test which showed very high rho coefficients for both allergen extracts ( $r=0.92$ ) and MA ( $r=0.96$ ) (Figure 1A, B). Using a Bland-Altman approach to test agreement between the two methods (Figure 1C, D), we observed a significant divergence ( $p < 0.0001$ ) between absolute differences only for values between 10 and 100 kU<sub>A</sub>/L, but not between residuals (difference/average). When considering values between 0.1 and 100 kU<sub>A</sub>/L, NOVEOS™ sIgE results were lower than ImmunoCAP™ results, by a mean of -15%, from -13% (extracts,  $p < 0.0001$ ) to -17% (MA,  $p = 0.0006$ ).

Then, we found a very good level of agreement ( $\kappa$  index 0.84; agreement 0.92) between the two methods (Figure E1). Highest or lowest levels of concordance were found for egg white extract ( $\kappa$  1.0, agreement 1.00), and for shrimp ( $\kappa$  0.74) and hazelnut extracts (agreement 0.81), respectively. Next and to gain further insight in the analysis of discordances, sIgE values were subdivided according to the sIgE reactivity classes from class 0 ( $\leq 0.35$  kU<sub>A</sub>/L) to classes 5-6 ( $> 50$  kU<sub>A</sub>/L) (Figure 2A). These classes allow sIgE analysis as discrete rather than continuous values (1). The percentage of agreement within

classes was 72% ( $n = 410/570$  sIgE results), while 23% (133/570) results differed by one class. Only 4.7% (27/570 results) differed by 3 or 4 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 (20/27) with mean sIgE values of 5.3 kU<sub>A</sub>/L (range: 0.1-23.4) for ImmunoCAP™ and 1.3 kU<sub>A</sub>/L (range: 0.13-7.6) for NOVEOS™. For low sIgE values, there was a 86% intermethod agreement for discrimination between sIgE classes “0” (<0.35kU<sub>A</sub>/L) and “1/2” (0.35 to 3.5kU<sub>A</sub>/L) (Figure 2A).

#### *Comparison of ImmunoCAP™ and NOVEOS™ clinical performance*

We performed ROC analysis to compare clinical performance of ImmunoCAP™ and NOVEOS™, and select sIgE optimal thresholds for discrimination between food allergic and non-allergic individuals. As presented in Table 2 and Figure E2, areas under ROC curves (AUC) varied from 0.79 for hazelnut extract (ImmunoCAP™ f17) to 0.97 for ovomucoid nGal d 1 (NOVEOS™ F233). The mean AUC was higher for NOVEOS™ than for ImmunoCAP™ ( $p=0.04$ ). Next, Youden’s index combining optimal sensitivity and specificity was used for setting the optimal sIgE cut-off values for the 10 food allergens (Table 2). Cut-off values for ImmunoCAP™ (mean  $6.6 \pm 3.9$  kU<sub>A</sub>/L) and NOVEOS™ (mean  $4.4 \pm 1.9$  kU<sub>A</sub>/L) were similar (paired samples Wilcoxon test,  $p=0.07$ ). The most divergent cut-off values were observed with hazelnut extracts (ImmunoCAP™ cut-off 16.7 kU<sub>A</sub>/L versus NOVEOS™ cut-off 3.6 kU<sub>A</sub>/L) and cow’s milk extracts (ImmunoCAP™ cut-off 6.9 kU<sub>A</sub>/L versus NOVEOS™ cut-off 3.3 kU<sub>A</sub>/L). Sensitivity, specificity, positive (PPV) or negative (NPV) predictive values were also comparable without significant difference (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 2). As some patients were under OIT at the sampling time, we analysed separately the 43 patients receiving peanut OIT (Table E3). When, considering only these patients, ROC curves had identical AUC values with both methods: 0.79 (peanut extract) and 0.84 (Ara h 2) (Table E3). Next, the ability of sIgE cut-offs to discriminate allergic from non-allergic individuals was evaluated by calculating OR, RR, Cohen’s kappa coefficients, and percentages of agreement between each method and patients’ status. A strong association was found between sIgE results for the 10 allergens and clinical status (Figure 3). The overall values of RR (ImmunoCAP™ 4.1 versus NOVEOS™ 4.6), OR, kappa 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 (Table E1). However, these differences were not significant. OR and RR were higher for MA with ImmunoCAP™ than with NOVEOS™, while agreement and kappa indexes were identical with both methods (Table E1). In both methods, MA were associated with better OR, RR, agreement and kappa indexes than allergen extracts (Table E1). Considering individual allergens (Table E2), cow’s milk sIgE results (extract and casein nBos d8) were associated with the highest RR (8.2), OR (>100), percentages of agreement (90-92%) and kappa indexes (0.80-0.83). The lowest values were obtained with ImmunoCAP™ peanut extract (RR: 2.7; kappa: 0.49; agreement: 75%) and NOVEOS™ hazelnut extract (RR: 2.6).

Cohen’s kappa index showed a “good” (0.61-0.80) or “very good” (0.81-1) degree of association for 6 out of 10 allergens tested for ImmunoCAP™ as compared to 8/10 for NOVEOS™ (Table E2,  $p=0.035$ , Wilcoxon  $t$ -test). In addition, agreement was higher than 80% for 9/10 allergens in NOVEOS™ and for 7/10 allergens in ImmunoCAP™ (Table E2). Overall, comparing kappa indexes and agreement for 10 allergens (*i.e.* 20 comparisons, Wilcoxon  $t$ -test:  $p<0.0001$ ), values were significantly higher for NOVEOS™ (11/20) than for ImmunoCAP™ (2/20).

Focusing on low sIgE values, contingency analyses were performed between class “0” ( $\leq 0.35$  kU<sub>A</sub>/L) and classes “1” or “2” (0.35 to 3.5 kU<sub>A</sub>/L) measurements, first for non-allergic (n= 234, Figure 2B), then for allergic patients (n=29, Figure 2C). The distribution of sIgE results for non-allergic patients differs between the two methods, with an excess of 31 ImmunoCAP™ class “1” or “2” results paired with class “0” NOVEOS™ results ( $p < 0.0001$ ). By contrast, no significant difference was found for allergic patients with classes “0” to “2” sIgE (Figure 2C).

#### *Origin of discordances between ImmunoCAP™ and NOVEOS™*

The 27 most divergent sIgE results from Figure 2A are shown in detail in Figure E3. As most discordant results concerned plant allergens (18/27), 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<sub>A</sub>/L; range 11 to  $> 100$  kU<sub>A</sub>/L, n = 12) were excluded from new ROC curves calculation. The new AUC were 0.81 for ImmunoCAP™ and 0.84 for NOVEOS™ (data not shown), compared to previous values of 0.79 for ImmunoCAP™ and 0.82 for NOVEOS™ before removal of the anti-Cor a 1 sIgE-positive samples. Cut-off values were unchanged (16.7 for ImmunoCAP™ versus 3.6 for NOVEOS™).

The second potential cause for discordant results resides in CCD displayed by plant allergens. We hypothesized that peanut or hazelnut positive results obtained using ImmunoCAP™ but not NOVEOS™ were related to IgE reactivity against CCD. We were able to test 11 samples (out of 25) for CCD IgE reactivity by using ImmunoCAP™ MUXF3 (o214) reagent, demonstrating anti-MUXF3 IgE in 8/11. We then used a reagent blocking CCD antibody reactivity (MUXF3-HSA, ProGlyCan reagent from Hämosan) for the three samples which were the most discordant for peanut (1 patient) and hazelnut extracts (2 patients). CCD-blocker reagent abolished ImmunoCAP™ reactivity against peanut and hazelnut extracts for these three samples, while testing these same samples with NOVEOS™ revealed no reactivity to peanut or hazelnut extracts, without adding the CCD-blocker (Table 3). In order to confirm these results in a different setting, we selected MUXF3-positive samples from 8 adult patients with *Hymenoptera* venom sensitization but without a history of FA. All these samples showed sIgE reactivity against ImmunoCAP™ hazelnut extract, but no or very low reactivity against NOVEOS™ hazelnut extract. The ImmunoCAP™ reactivity was abolished or strongly reduced after addition of the CCD-blocker reagent (Table 3).

## 284 Discussion

285 We report here the compared performance of two sIgE platforms and their clinical cut-offs for 10  
 286 common food allergens. Clinical cut-offs for sIgE have been previously proposed multiple times, in  
 287 particular for food allergens, as indicators of the probability of presenting with allergic symptoms,  
 288 rather than thresholds accurately predicting the occurrence of symptoms (15, 27, 28). Thus, the  
 289 quantitative nature of sIgE measurements is essential for allergy diagnosis, and physicians must be  
 290 aware of the characteristics of the employed methods. Indeed, several routine methods of sIgE  
 291 quantitation co-exist, belonging to successive generations and to different times of availability for  
 292 clinical use. First generation tests were radioimmunoassays which used allergens bound to paper disks  
 293 and an anti-IgE reagent labelled with a radio-isotope, usually  $^{125}\text{I}$ : the RAST (RadioAllergoSorbent Test,  
 294 Pharmacia Diagnostics AB, Uppsala, Sweden) was commercialized in 1974 (29). The main second-  
 295 generation test, ImmunoCAP<sup>TM</sup> (Pharmacia AB, now ThermoFisher Scientific), has allergens covalently  
 296 bound to a nitrocellulose sponge and uses fluorescent signals for the quantitation of anti-IgE (4). Third  
 297 generation sIgE tests are represented by the IMMULITE 2000 system (Siemens Healthcare SAS, Saint-  
 298 Denis, France), with biotinylated soluble allergens binding to a large (25mm diameter) avidin-coated  
 299 unique bead, and chemiluminescent signals for anti-IgE binding (30). Recently (2020), fourth-  
 300 generation technologies for sIgE determination became available (NOVEOS<sup>TM</sup>, Hycor, Garden Grove,  
 301 CA, USA and IDS-iSYS, Bolton, UK), differing from third generation tests through the use of biotin-  
 302 labelled allergens bound to avidin micro-beads (17, 31).

303 Here, we found that NOVEOS<sup>TM</sup> sIgE results were significantly lower than those obtained with  
 304 ImmunoCAP<sup>TM</sup>, by a mean value of 15%. This discrepancy is not due to a defect in the linearity of  
 305 NOVEOS<sup>TM</sup> technology (32) which is similar to that of ImmunoCAP<sup>TM</sup> (31). The differences we observed  
 306 between the two methods could be due to an underestimation by NOVEOS<sup>TM</sup>, to an overestimation by  
 307 ImmunoCAP<sup>TM</sup>. An underestimation by NOVEOS<sup>TM</sup> could be due to a lower density of allergen molecules  
 308 on NOVEOS<sup>TM</sup> beads compared to ImmunoCAP<sup>TM</sup> solid phase. Significant discrepancies had been  
 309 reported previously between ImmunoCAP<sup>TM</sup> and Immulite<sup>TM</sup> (33). In our hands, significant analytical  
 310 differences were observed mostly for higher ( $>3.5 \text{ kU}_A/\text{L}$ ) sIgE concentrations (Figure 1C, D). The clinical  
 311 significance of these discrepancies is probably very low, especially for sIgE measurements greater than  
 312 the clinically relevant cut-offs.

313 By contrast, in sensitized individuals having low sIgE values ( $\leq 0.35$  to  $3.5 \text{ kU}_A/\text{L}$ ; IgE reactivity classes 0  
 314 to 2), we found a better NPV with NOVEOS<sup>TM</sup> (Figures 2B, C). Correct quantification of low levels  
 315 of sIgE is important for early detection of sensitization against food allergens in children (34, 35). These  
 316 results need to be confirmed by further prospective studies.

317 We also investigated two potential causes for some false positive results for peanut and hazelnut  
 318 extracts in non-allergic patients tested with ImmunoCAP<sup>TM</sup>: Cor a 1 spiking of ImmunoCAP<sup>TM</sup> hazelnut  
 319 extract and the presence of CCD on plant allergen extracts. While Cor a 1 spiking of ImmunoCAP<sup>TM</sup>  
 320 hazelnut extract did not contribute to clinical performance discrepancies, CCD did. Thus, our study  
 321 supports the view that glycosylated epitopes are more accessible to sIgE with ImmunoCAP<sup>TM</sup> than with  
 322 NOVEOS<sup>TM</sup>. This could be due to the avidin-coated beads and the biotinylation of NOVEOS<sup>TM</sup> allergens.  
 323 Another possibility is that anti-CCD sIgE react both with CCD determinants on allergen molecules and  
 324 also with the nitrocellulose sponge matrix on ImmunoCAP<sup>TM</sup> (36, 37). Unlike the animal-derived  
 325 galactose- $\alpha$ -1,3-galactose epitope, plant CCD (*e.g.* MUXF3), are currently considered devoid of clinical

relevance in allergy (38). This could explain the better correlation between plant FA and NOVEOS™ sIgE results. As expected, recombinant, non-glycosylated MA displayed similar clinical performance.

Despite these discrepancies, we report that NOVEOS™ and ImmunoCAP™ have mostly similar performance for discriminating between food allergic and non-allergic individuals. It is impossible to determine clinically relevant universal thresholds of sIgE concentrations due to important variations from one population to another. For example, the proposed cut-off for rAra h 2 sIgE concentration in peanut allergy varies from 0.10 to 42.2 kU<sub>A</sub>/L (16). However, the establishment of “local” clinical cut-offs is of utmost importance for the management of 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<sub>A</sub>/L with 95% specificity and 44% sensitivity, which was similar to values reported in this 15-20-year later study (cut-off 14kU<sub>A</sub>/L, 86% specificity and 51% sensitivity) (28). Thus, cut-off values can be established for a given population on the condition of using similar protocols and appear stable over time. Our study further supports that sIgE measurements by either ImmunoCAP™ or NOVEOS™ are highly informative on the risk of allergy, with OR >10 and RR >2 in our population.

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

In conclusion, 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 ten-fold lower test sample volume (4 µL) compared to ImmunoCAP™ (40 µL), which can be advantageous for allergy diagnosis in children. Further confirmatory studies are warranted including more allergens (*i.e.* other food allergens, respiratory, venom, drugs) and both adult and pediatric patients from other geographical areas. However, allergology practitioners must remember that regardless of the method used for specific IgE measurements, these methods are tools aimed at improving the physician’s clinical analysis and decision, but are not sufficient by themselves to establish an allergy diagnostic.

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| allergen   | Number of patients | median age (IQR) | sex ratio | before FA testings              |                    |                                     | after FA testings                |                         |
|------------|--------------------|------------------|-----------|---------------------------------|--------------------|-------------------------------------|----------------------------------|-------------------------|
|            |                    |                  |           | strict avoidance <sup>(1)</sup> | OIT <sup>(2)</sup> | episodic consumption <sup>(3)</sup> | patients with OFC <sup>(4)</sup> | allergic <sup>(5)</sup> |
| 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                   |

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**TABLE 1** Demographics and clinical status of patients.

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FA testings (n=337) were conducted in 289 patients; 48/289 individuals presented with 2 FA were tested for the corresponding allergens. <sup>(1)</sup> patients following a strict avoidance diet. <sup>(2)</sup> patients receiving OIT for the tested food at time of sampling. <sup>(3)</sup> patients not following OIT but consuming the culprit food episodically. <sup>(4)</sup> patients with a FA diagnosis based on OFC. <sup>(5)</sup> patients with a confirmed FA diagnosis. FA, food allergy.

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| Allergen, code <sup>(1)</sup> | Type <sup>(2)</sup> | Method <sup>(3)</sup> | AUC  | Optimal Cut-Off sIgE level (kU <sub>A</sub> /L) | Sensitivity | Specificity | PPV  | NPV  | sIgE level for $\geq 95\%$ PPV (kU <sub>A</sub> /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 <sup>(4)</sup>                                 |
| egg white, F001               | E                   | N                     | 0.90 | 6.5                                             | 93%         | 72%         | 63%  | 95%  | 26.7 <sup>(4)</sup>                                 |
| 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                                                |

476

477 **TABLE 2** Summary of ImmunoCAP™ and NOVEOS™ clinical performance.

478 Cut-off values (sIgE kU<sub>A</sub>/L) correspond to optimal correlations between sIgE values and FA diagnosis  
 479 (highest Youden index). PPV, NPV: positive / negative predictive values. sIgE concentrations associated  
 480 with PPV above 95% are in kU<sub>A</sub>/L. <sup>(1)</sup> Allergen source and identification; <sup>(2)</sup> E: allergen extract, M:  
 481 molecular; <sup>(3)</sup> I: ImmunoCAP™ (ThermoFisher), N: NOVEOS™ (Hycor); <sup>(4)</sup> maximum PPV = 90%.

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| Sample # | Age (y) | Sex | MUXF3 <sup>(1)</sup> | Allergen extract | ImmunoCAP™  | NOVEOS™             |         |
|----------|---------|-----|----------------------|------------------|-------------|---------------------|---------|
|          |         |     |                      |                  | CCD-blocker |                     |         |
|          |         |     |                      |                  | without     | with <sup>(2)</sup> | without |
| 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   |

485

486 **TABLE 3** MUXF3-positive samples show reactivity against peanut or hazelnut extracts with  
 487 ImmunoCAP™ f13/f17 reagents, but not with NOVEOS™ F013/F017.

488 Samples #1-3 were chosen for their discordance for peanut or hazelnut extract: positive with  
 489 ImmunoCAP™ versus negative/very low for NOVEOS™. Patients #4-11 are not from the main study and  
 490 were selected for their MUXF3-positivity during routine testing for sensitization against Hymenoptera  
 491 venom. All values are in kU<sub>A</sub>/L <sup>(1)</sup> MUXF3 sIgE were measured using ImmunoCAP™ o214; <sup>(2)</sup> CCD-blocker  
 492 reagent (MUXF3-HSA) was added.  
 493

494

**FIGURE 1** Quantitative correlation between ImmunoCAP™ and NOVEOS™.

A, C: allergen extracts (337 data points); B, D: molecular allergens (233 data points); r: Spearman's rho; C, D: Bland-Altman diagrams plotting differences (ordinate values, in KU<sub>A</sub>/L) in sIgE measurement between NOVEOS™ and ImmunoCAP™, against the average values (abscissa). Ninety-six results exceeded the methods' LODs (72 below 0.1/0.17 and 24 above 100kU<sub>A</sub>/L) and were replaced by their respective LOD values.

**FIGURE 2** Semi-quantitative correlation between ImmunoCAP™ and NOVEOS™.

**A:** Specific IgE results from both methods were grouped into IgE reactivity classes and the number of data points is indicated for all combinations of NOVEOS™ and ImmunoCAP™ results; red: allergic patients; blue: non-allergics; circles: extracts; squares: MA. **B, C:** sIgE results (only classes 0, 1 or 2) contingency tables and Fisher's exact tests for non-allergic (B) or allergic patients (C).

**FIGURE 3** Relative risk (A), odds-ratio (B), kappa index (C) and percentage of agreement (D) of clinical allergy assessed from sIgE levels.

Individuals were categorized according to their FA or food tolerance and to the sIgE cut-off for each allergen. OR and RR of FA were calculated from contingency tables. ImmunoCAP™: red circles; NOVEOS™: blue squares; molecular allergens are underlined; dashed lines show thresholds for clinical pertinence: RR>2, OR>10, kappa index rankings for the degree of agreement with clinical status and percentages of agreement above 80%.

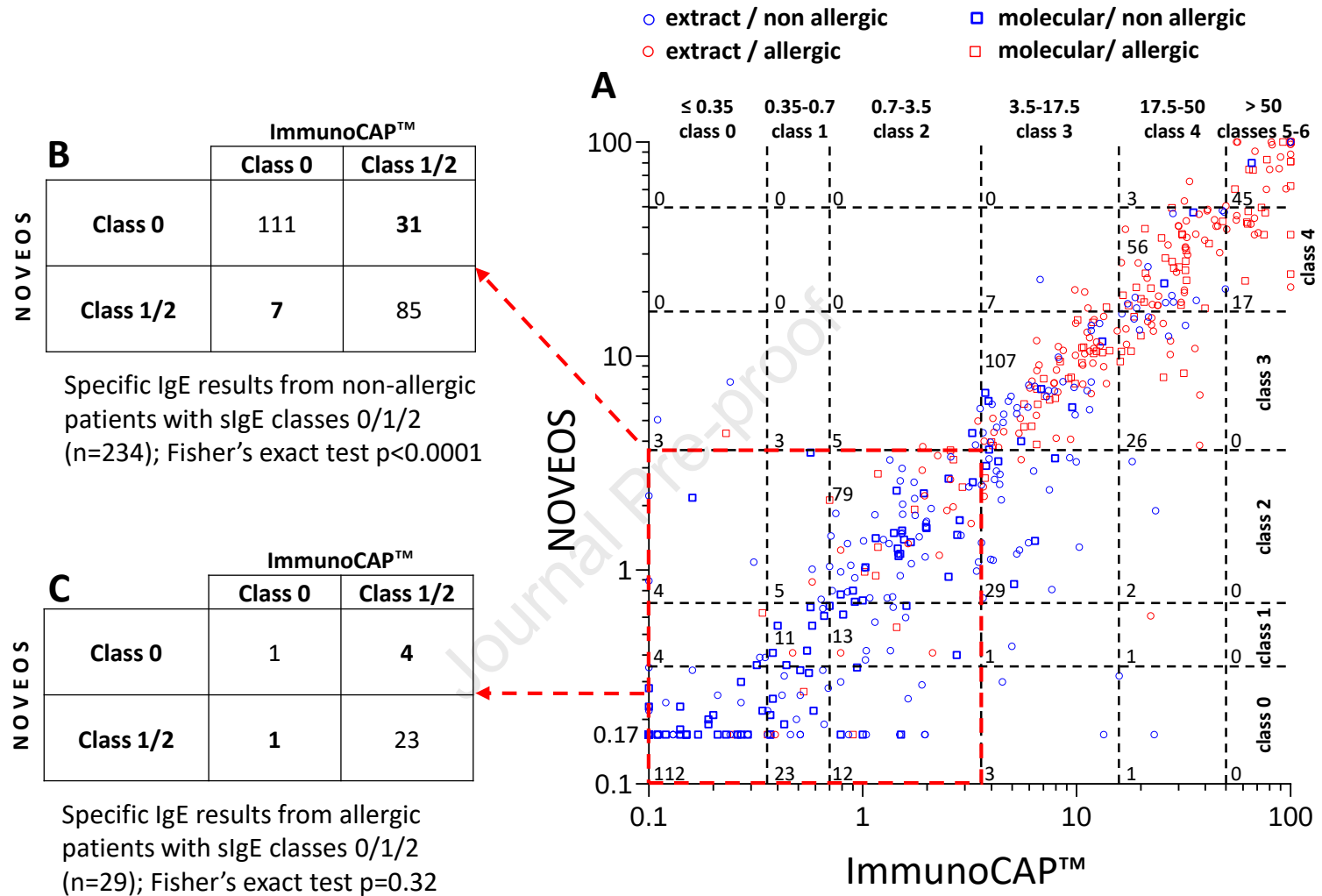
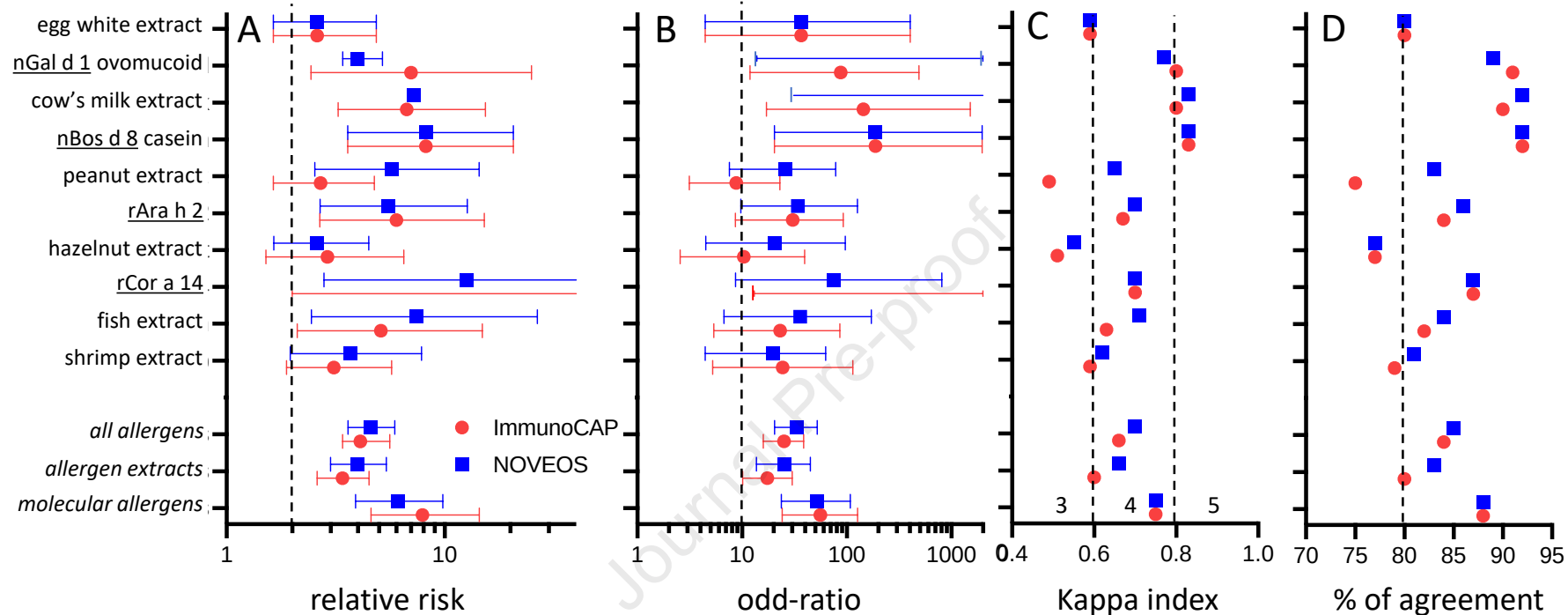


Fig. 2



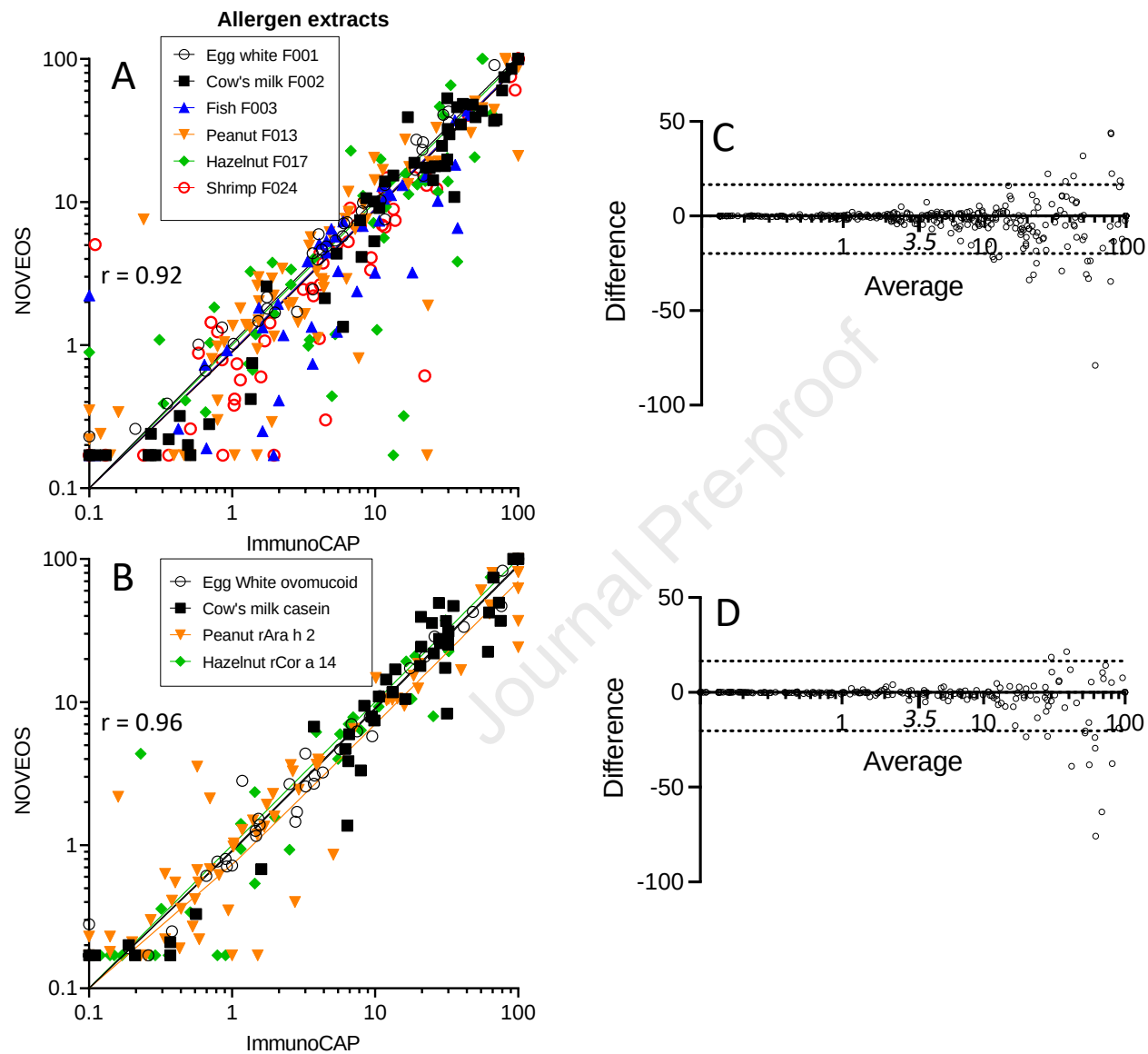


Fig. 1