NOVEOS (Hycor) demonstrates better clinical performance than ImmunoCAP (Thermofisher) for food allergy diagnosis

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Abstract

Background: The aim of this study was to compare the technical and clinical effectiveness of two platforms (Phadia ImmunoCAP and Hycor NOVEOS) for the measurement of IgE specific for 10 food allergens. **Methods:** 289 patients, as part of allergy diagnosis or of their follow-up were included and 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 the ImmunoCAP and NOVEOS methods. Food allergy diagnosis was established according to international guidelines. **Results:** A very good 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 provided higher overall odd-ratios and relative risks for allergen extracts than ImmunoCAP, but the difference was not significant. When all ten allergens were considered, NOVEOS provided better ROC curves (p=0.03) and thus, had a better ability to establish the true value. 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 (odd-ratios of allergy = 25.1) or NOVEOS (odd-ratios of allergy = 33.0) is similarly 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 IgE specific for carbohydrate determinants, while requiring a ten-fold lower test sample volume.

Introduction

Determination of specific IgE (sIgE) is one of the pillars on which allergy diagnosis stands, 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* utilizing automated systems. IgE sensitization is commonly demonstrated *in vivo* by skin prick testing (SPT), or *in vitro* utilizing 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 many factors such as variations in the composition of the allergenic sources (6), diversity of 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 impact of these tests has been restricted to the correct identification of allergen(s) responsible for clinical symptoms and to the estimation of the risk of severe reactions, deduced from the sensitization profile against MA (12-14). 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 (15).

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 thus, diverge from ImmunoCAP, which is based on allergens bound to a cellulose matrix and fluorescence signal. 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 (16) 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 (17).

However, comparison of the clinical performance of the two methods has not been addressed yet, and data for food allergens is lacking. The main objective of this study was to determine whether the theoretical technical advantages of NOVEOS technology can be translated into an equivalent or superior performance to that of ImmunoCAP, in a clinical setting of food allergy.

Materials and methods

Patients and ethical considerations

Patients (n=289) were recruited between 2017 and 2021 from paediatric or adult pulmonology and allergology departments in the Toulouse Teaching Hospital. All patients were attending for a suspicion of allergy to one or several foods. In all patients, food allergy diagnosis was established based on open oral food challenge (OFC) and/or anamnesis and the demonstration of sensitization to culprit foods. Blood samples were taken as part of routine allergy diagnosis or follow-up, in agreement with current EAACI and WAO guidelines (18, 19). The present study concerned only previously generated clinical and laboratory data and additional experiments with excess serum, and was thus was categorized as a type 3b non–interventional research, Art. L1121-1 CSP under the French law. The study was approved by Research Ethics Committee (*Comité de Protection des Personnes*) Sud-Ouest et Outre-Mer II, for samples collected in Toulouse Teaching Hospital (sample collection declaration DC20162804). Sera from eight patients exhibiting MUXF3-positive sIgE without a history of food allergy, distinct from the 289 patients described above, were also selected.

Oral food challenges (OFC)

Open (single-blind) food challenges were supervised by trained practitioners using recommended threshold cumulative doses (20). Due to risk of severe anaphylaxis or refusal, only 59% of patients were investigated with OFC (range: 32% for shrimp-allergic to 100% for peanut-allergic patients). 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), cow's milk (>8.5 oz/254 mL of raw milk), 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). Ongoing oral immunotherapy (OIT) was not considered as an exclusion criterion, as 31% of patients were receiving OIT at time of inclusion.

Specific IgE measurements

Specific IgE measurements were performed with both ImmunoCAP Phadia 250 (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) and NOVEOS (Hycor, Garden Grove, CA, USA) systems, in full compliance with NF EN ISO 15189 standards (certification #8-1769). Following the initial determination by the ImmunoCAP method, samples were kept frozen at -40degC before testing with NOVEOS. For statistical analyses, sIgE values outside of the analyzers' ranges of measurement (ImmunoCAP: 0.10 - 100kU$_A$/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 >100 kU$_A$/L. Some samples were tested after addition of a CCD-blocker reagent (ProGlyCan MUXF3-human serum albumin, Hamosan, 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, and percentage of agreement (proportion of both true positive and true negative results). Clinical performance of specific IgE values was determined through odd-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, and percentages of agreement. 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). All statistical calculations were performed by using PRISM 9 (GraphPad Software, San Diego, California USA,*www.graphpad.com*). Significance was set at $p>0.05$. Optimal cut-off of sIgE values were calculated using Youden's index (21).

Results

Patients characteristics

The study population (183/289 were males; 63%) displayed a median age of 7 years, and 246/289 (85%) were aged 15 years or younger (Table 1). In all patients, the food allergy diagnosis was established on the basis of open OFC and/or anamnesis. The percentage of confirmation for suspected food allergy in the cohort ranged from 34% (egg white) to 52% (cow's milk). Confirmed allergy to multiple foods (n>2) was reported in 48/289 (17%) patients.

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/ovomucoid (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$_A$/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, $p<0.0001$) to -17% (MA, p=0.0006).

Then, we found a very good level of agreement ($\varkappa$ index 0.84; agreement 0.92) between the two methods (Figure S1). Highest or lowest levels of concordance were found for egg white extract ($\varkappa$ 1.0, agreement 1.00), and for shrimp ($\varkappa$ 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 ([?]0.35 kU$_A$/L) to classes 5-6 (>50 kU$_A$/) (Figure 2A). 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 results) differed by 3 to 4 IgE reactivity classes and corresponded to 25 patients (two patients were discordant for both extract and MA).

Comparison of ImmunoCAP and NOVEOS clinical performance

We performed ROC analysis to compare clinical performance of ImmunoCAP and NOVEOS. We also selected sIgE optimal thresholds allowing optimal discrimination between food allergic and non-allergic individuals. As presented in Table 2 and Figure S2, areas under ROC curves (AUC) were comprised from 0.79 for hazelnut extract (ImmunoCAP f17) to 0.97 for ovomucoid nGal d 1 (NOVEOS F233). However, the mean value of AUC was higher for NOVEOS than for ImmunoCAP (p=0.03). Next, and for setting the optimal threshold for the 10 food allergens, Youden's index that combines optimal sensitivity and specificity was calculated and the optimal cut-off values for sIgE reported in Table 2. Cut-off values for ImmunoCAP and NOVEOS were similar (mean: 6.7 ± 3.8 kU_A/L for ImmunoCAP and 4.6 +- 2.1 kU_A/L for NOVEOS) and the difference was not significant. The most divergent cut-off values were between hazelnut extract (ImmunoCAP cut-off 16.7 kU_A/L versus NOVEOS cut-off 3.6 kU_A/L) and cow's milk (ImmunoCAP cut-off 6.9 kU_A/L versus NOVEOS cut-off 3.3 kU_A/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). Next, sIgE cut-offs established for the 10 food allergens were further used to discriminate allergic from non-allergic individuals by calculating OR, RR, Cohen's kappa coefficients, and percentages of agreement between each method and patients' status. As presented in Figure 3, a strong association between sIgE results and clinical status was reported for the 10 allergens. The overall values of RR (4.1 for ImmunoCAP versus 4.6 for NOVEOS), 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 S1). However, these differences were not significant. For the four MA, OR and RR were higher for ImmunoCAP than for NOVEOS, while agreement and kappa indexes were identical with both techniques (Table S1). In addition, both techniques were associated with better OR, RR, agreement and kappa indexes for MA than for allergen extracts (Table S1). Considering individual allergens (Table S2), 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 hazelnut extract (RR: 2.6 for NOVEOS).

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 S2, p=0.035, Wilcoxon test). In addition, percentages of agreement were above 80% for 9 out of 10 allergens tested with NOVEOS while this was true for 7 out of 10 allergens tested with ImmunoCAP (Table S2). Overall, comparing kappa indexes and agreement for 10 allergens (*i.e.* 20 comparisons), values were more frequently higher for NOVEOS (11/20) than for ImmunoCAP (2/20).

Finally, thirty-one non-allergic patients present with a class "1" or class "2" sIgE results (0.35 to 3.5 kU_A/L) with ImmunoCAP, but have a class "0" ([?]0.35 kU_A/L) NOVEOS result (p<0.0001, Figure 2B), while there is no significant difference between the two methods for allergic patients with low sIgE results (Figure 2C).

Origin of discordances between ImmunoCAP and NOVEOS

The 27 most divergent sIgE results from Figure 2A are shown in detail in Figure S3. 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 (22). 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 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 o214 reagent and found that 8/11 samples possessed anti-MUXF3 IgE. We then used a reagent blocking CCD antibody reactivity (MUXF3-HSA, ProGlyCan reagent from Hamosan) 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 food allergy. 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).

Discussion

We report here the compared performance of two sIgE platforms and their clinical cut-offs for 10 common food allergens. Clinical cut-offs for sIgE have been previously 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 (14, 23, 24). Thus, the quantitative nature of sIgE measurements is essential for allergy diagnosis, and physicians must be aware of the characteristics of the employed methods. Indeed, several routine methods of sIgE quantitation co-exist, because they belong to successive generations and to different times of availability for clinical use. First generation tests were radioimmunoassays which used an anti-IgE reagent labelled with a radio-isotope, usually ^{125}I: the RAST (*RadioAllergoSorbent Test, Pharmacia Diagnostics AB, Uppsala, Sweden*) was commercialized in 1974 (25). The main second-generation test is based on the ImmunoCAP technology (originally from Pharmacia AB, now ThermoFisher Scientific), where allergens are covalently attached to a nitrocellulose sponge (4). Third generation sIgE tests are represented by the IMMULITE 2000 system (Siemens Healthcare SAS, Saint-Denis, France), which uses biotinylated soluble allergens bound to a large diameter (25mm) avidin-coated unique bead, and a chemiluminescent signal is used for detection (26). Recently (2020), fourth-generation technologies for sIgE determination were made available (NOVEOS, Hycor, Garden Grove, CA, USA and IDS-iSYS, Bolton, UK), differing from third generation tests through the use of biotin-labelled allergens bound to avidin micro-beads and chemiluminescent detection (16, 27).

We found that NOVEOS sIgE results were significantly lower than those obtained with ImmunoCAP, by a mean value of 15%. This discrepancy is not due to a defect in the linearity of NOVEOS technology (28) which is similar to that of ImmunoCAP (27). The differences we observed between the two methods could be due to an underestimation by NOVEOS, to an overestimation by ImmunoCAP, or both. Significant discrepancies had been reported previously between ImmunoCAP and Immulite, a third-generation technology developed by SIEMENS (29). It is notable that our data show very few differences for low values (0.1 to 1 kU_A/L). These levels of sIgE are important for early detection of sensitization against food allergens in children (30, 31). By contrast, if the two methods produce significantly different results for high sIgE concentrations, these discrepancies are of lesser clinical significance, especially if they are greater than the clinically relevant cut-offs.

For clinical performance, NOVEOS is better able to exclude allergy in sensitized individuals having low sIgE values (0.35-3.5 kU_A/L) and we also found some false positive results in non-allergic patients tested with ImmunoCAP for peanut and hazelnut extracts. Despite these discrepancies, we report that NOVEOS and ImmunoCAP have mostly similar performance for discriminating between food allergic and food tolerant individuals. It is impossible to determine clinically relevant universal thresholds of sIgE concentrations due to important variations from one population to another one. For example, the cut-off for rAra h 2 sIgE concentration in peanut allergy varies from 0.10 to 42.2 kU_A/L between studies (15). However, the

establishment of "local" clinical cut-offs is of utmost importance for the management of a given population of patients 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 (cut-off of 15kU$_A$/L with 95% specificity and 44% sensitivity) which was similar to the values we report here (cut-off of 14kU$_A$/L, 86% specificity and 51% sensitivity) (24). Thus, cut-off values can be established for a given population on the condition of using similar protocols and seem to be stable for extended periods (20 years in this example). Our study further supports that sIgE measurements by both ImmunoCAP and NOVEOS, are highly informative on the risk of allergy in the patients we studied based on OR values >10 and RR>2.

We investigated two potential causes of discrepancies in clinical performance between NOVEOS and ImmunoCAP with hazelnut and peanut extracts, namely Cor a 1 spiking of ImmunoCAP hazelnut extract and the presence of CCD. While 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 both with CCD determinants on allergen molecules and also with the nitrocellulose sponge matrix on ImmunoCAP (32, 33). Unlike the animal-derived galactose-α-1,3-galactose epitope, plant CCD (*e.g.* MUXF3), are currently considered devoid of clinical relevance in allergy (34). This could explain our findings of better sIgE - confirmed plant food allergy correlation with NOVEOS than with ImmunoCAP. For recombinant MA which are non-glycosylated in both systems we found similar clinical performance as expected.

There are several limitations to our study. Firstly, this is a retrospective, monocentric study. Secondly, the population was mainly comprised of children (85%). In addition, and depending on the allergen, the percentage of patients under a strict avoidance diet varied from 12% (peanut) to 53% (seafood) and an OFC was not systematically performed for food allergy diagnosis, except for peanut. On the other hand, our study encompasses a large number of comparisons including both extracts and molecular food allergens. Moreover, the heterogeneity of the patients and of their therapeutic protocols mirrors our regular clinical practice.

In conclusion, we demonstrate here that, for 10 common food allergen extracts and molecules, assayed in a large pediatric cohort, sIgE determination performed with NOVEOS or with ImmunoCAP are highly correlated with and predictive of the actual diagnosis of food allergy or tolerance. Despite a ten-fold lower test sample volume requirement (4 µL) compared to ImmunoCAP (40 µL), NOVEOS has an overall better capacity to identify patients at risk of allergy versus asymptomatic sensitization (p=0.03 when AUC are compared). 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.

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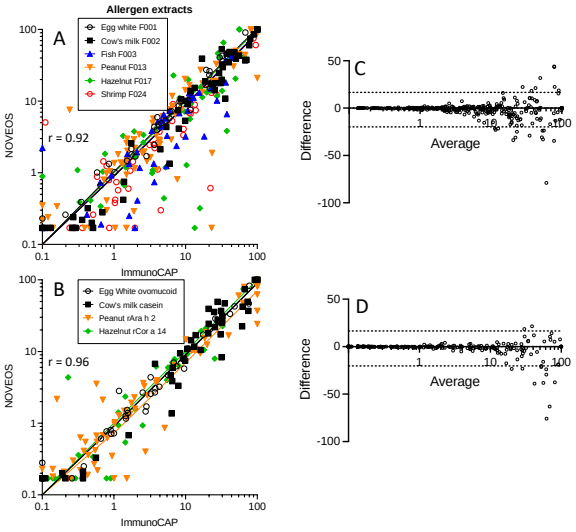

FIGURE 1

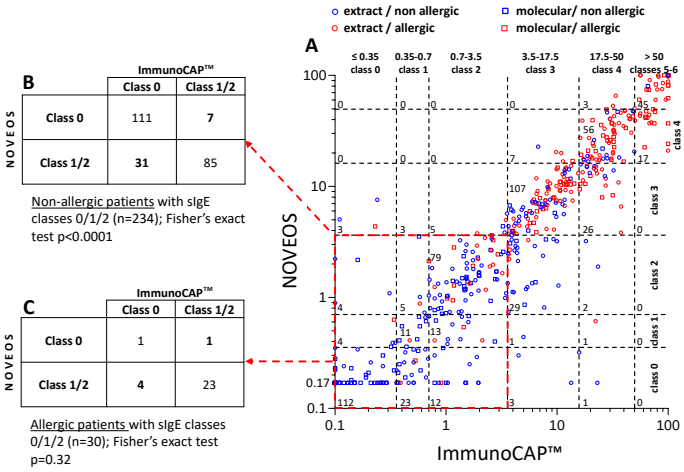

FIGURE 2

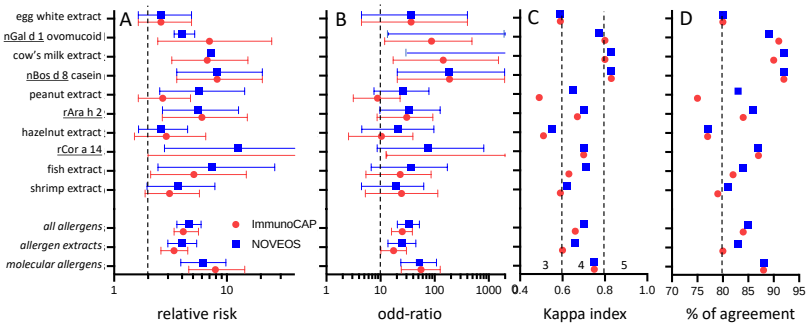

FIGURE 3

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