
Extension of food allergen specific IgE ranges from the ImmunoCAP to the IMMULITE systems

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Background: Quantitative measures of food specific IgE antibody using the Phadia ImmunoCAP method have documented value in the diagnosis of food allergy.

Objective: To define factors that could be used to relate IMMULITE-measured IgE antibody levels for chicken egg white, cow's milk, and peanut into ImmunoCAP-comparable quantities that could then be correlated with published levels that have been generated with the ImmunoCAP system.

Methods: Serum samples from 328 patients (median age, 5.4 years; age range, 1–18 years; 32% female) who were known to be IgE positive (>0.1 kU/L) to chicken egg white ($n = 120$), cow's milk ($n = 135$), and/or peanut ($n = 304$) were analyzed in both the ImmunoCAP and IMMULITE autoanalyzers.

Results: IgE antibody levels from both assays for each of the 3 food specificities were highly correlated: $r^2 = 0.95$ for egg white, $r^2 = 0.93$ for milk, and $r^2 = 0.95$ for peanut ($P < .001$). Empirically determined IMMULITE/ImmunoCAP ratios (mean $\pm$ 1 SD) were 4.85 ± 1.79 kU/L (egg), 2.33 ± 1.0 kU/L (milk), and 1.86 ± 0.98 kU/L (peanut). For milk and peanut, the IgE antibody levels for individuals who either passed or failed a food challenge were not significantly different between the assay methods. Because of the small sample size of egg white challenged patients, no statistical analysis was performed.

Conclusion: These data indicate that specific IgE levels to egg white, milk, and peanut measured by the IMMULITE and ImmunoCAP systems are highly correlated and that differences between the systems are circumscribed and modest (IMMULITE was a mean of 2- to 5-fold higher than ImmunoCAP).

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INTRODUCTION

From the moment that reagenic activity was linked to IgE by the World Health Organization (WHO),¹ the allergy community has desired a biomarker that could predict the presence of symptomatic allergic disease in an individual exposed to an allergen. Unfortunately, it is well established that the presence of IgE antibody is necessary but not sufficient for symptomatic allergic disease. The presence of IgE antibody signifies sensitization to an allergen that can be viewed as a risk factor. It does not, however, ensure that the patient will manifest allergic symptoms on allergen exposure. Additional host and allergen/environmental factors complicate this relationship. Thus, the early radioallergosorbent assay that permitted detection of IgE antibody was certainly a technologic

advance. However, although serologic and skin test measures of IgE antibody have remained useful as biomarkers of the predisposition for allergic disease, their results must be carefully interpreted within the context of the patient's clinical history.

Advances in assay technology have ushered in the current generation of autoanalyzers, which provide more precise quantification of IgE antibody levels in serum.² Microprocessor-driven instrumentation in particular has brought with it tighter intra-assay precision and interassay reproducibility. Calibration against the WHO 75/502 Reference IgE Standard has allowed present-day autoanalyzers to achieve a high degree of accuracy with regard to quantifying allergen specific IgE antibody. Using the enhanced quantitative capability of the ImmunoCAP autoanalyzer, studies from 1997³ and 2001⁴ demonstrated that absolute values of food specific IgE antibody concentrations could better identify patients who were not just sensitized but more likely truly allergic to egg, milk, peanut, or fish. Subsequent studies have essentially confirmed the predictive power principle of IgE antibody serologic testing for these select foods, but they have reported different predictive cut points, largely because of differences in the study populations and the method of data analysis. To date, all the analyses reported in the literature that relate to predicting symptomatic food allergy have been performed with the ImmunoCAP system. This has led to questions about

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how to use IgE antibody levels from other assays, such as the IMMULITE autoanalyzer, in the diagnosis of symptomatic food allergy.

To extend the utility of the serologic measurements of IgE antibody, we performed a study to examine the relationship between the quantitative results generated with the ImmunoCAP and the IMMULITE systems. Although we confirm the previous observation that the 2 methods report different levels of IgE antibody for a given specificity, the magnitude of this difference has not been previously examined in detail. In this study, we focus on 3 foods (chicken egg white, cow's milk, and peanut) to examine the relationship between the 2 assay systems. The goal of this study was to define a factor that could be used to relate IMMULITE-measured IgE antibody levels for egg white, milk, and peanut into ImmunoCAP-comparable quantities that could then be correlated with published levels that have been generated with the ImmunoCAP system.

METHODS

Study Population

Serum samples from a total of 328 patients were obtained from banked excess serum on which patients had given their verbal consent for IgE antibody testing. Most of the serum samples were originally drawn for clinical purposes from patients visiting the Johns Hopkins Pediatric Allergy Clinic. A subset of the patients providing serum samples had multiple sensitivities to egg, milk, and peanut. Human subjects approval was provided by the Johns Hopkins institutional review board. Most of the food challenges that were performed on patients providing serum samples to this study were open and performed for diagnostic purposes during a clinic visit. The cumulative maximum food challenge dose was 5 g of protein for egg white, cow's milk, or peanut for children younger than 5 years and 8 g otherwise. In addition, serum samples were also used from a subset of 23 patients who participated in a milk oral immunotherapy study and all failed a double-blind, placebo-controlled milk challenge. Thus, 2 distinct groups of patients were identified for analysis: children who had undergone a diagnostic open food challenge (OFC) for egg white, milk, or peanut in a clinical setting or a placebo-controlled milk challenge as part of a milk oral immunotherapy study (group A) and children with a history of serious allergic reaction to egg, milk, or peanut or with a markedly elevated IgE level who could not be ethically challenged (group B). All serum samples from patients selected for the study had detectable egg white ($n = 120$), milk ($n = 135$), and/or peanut ($n = 304$) specific IgE levels greater than 0.1 kU/L. Insufficient numbers of serum samples from patients with egg allergy could be identified who had undergone an OFC to ensure statistical validity; thus, no data are provided from challenged egg allergic patients.

Serologic Analyses

Egg white, milk, or peanut specific IgE antibodies were quantified by the ImmunoCAP 250 (Phadia, Uppsala, Sweden) and the IMMULITE 2000 (Siemens Healthcare Diagnostics, Tarrytown, New York) systems as per the manufacturers' assay protocols. The serum requirements per allergen specific IgE analysis were 40 μ L (ImmunoCAP) and 50 μ L (IMMULITE). The 2 assay systems differ in the solid-phase matrix used (ligand particle for IMMULITE vs sponge for ImmunoCAP), method of immobilizing allergen on the solid phase (ligand receptor interaction for IMMULITE vs direct covalent coupling for ImmunoCAP), source of allergens used to manufacture their reagents, and the method used to establish the calibration curve (electronically stored manufacturer-determined calibration curve with user run internal adjusters for IMMULITE vs user run calibration curve that is electronically stored for a predefined period for ImmunoCAP). Internal controls were performed in each assay run and were required to be within 2 SDs of the tolerance ranges to accept each assay. When the interpolated IgE antibody concentration exceeded the top point of the calibration curves (eg, >100 kU/L), the specimen was diluted with an appropriate serum diluent and reanalyzed to obtain a quantitative measure of antibody in that specimen.

Statistical Analyses

Categorical variables were computed and regression analyses were performed using the statistics portion of SigmaPlot-11 (Systat Software Inc, San Jose, California). The significance of the intermethod differences in levels of IgE antibody for the patients who passed or failed their milk or peanut OFCs was assessed using the Mann-Whitney rank sum test.

RESULTS

Correlation of the ImmunoCAP and IMMULITE Measurements

Table 1 summarizes the demographics of the study patient groups. Serum samples were collected from patients (median age, 5.4 years; range, 1–18 years; 32% female) who had either undergone an OFC for their respective food ($n = 122$) or who could not be challenged because of a definitive history of a severe allergic reaction or a markedly elevated IgE level ($n = 437$) to egg, milk, or peanut. Figures 1, 2, and 3 display the correlation of IgE antibody results specific for egg white, milk, and peanut, respectively, as measured by ImmunoCAP (abscissa) and IMMULITE (ordinate) in the combined set of serum samples from the challenged and unchallenged groups. With all 3 specificities, the intermethod agreement was highly correlated ($P < .001$) between the ImmunoCAP and IMMULITE systems using log-log plots, with regression line slopes for all 3 specificities approaching unity. Figures 2 and 3 differentially display the levels of IgE antibody obtained from patients who passed (closed circles) or failed (open circles) an OFC.

Table 1. Study Group Demographics

Group	Challenge result	No. of patients	Age range, y	IgE antibody ImmunoCAP range (median), kU/L	IgE antibody IMMULITE range (median), kU/L
Egg					
History positive, not challenged		120	1–16	0.45–536.1 (21.9)	1.48–2370 (98.7)
Milk					
OFC	Passed	6	1–18	0.18–3.43 (0.62)	0.15–5.30 (1.15)
OFC	Failed	3	1–12	0.17–1.19 (0.82)	0.66–2.31 (1.65)
DBPCFC	Failed	22	1–15	4.26–572 (38.7)	3.60–1290 (87.5)
History positive, not challenged		104	1–18	0.35–1634 (38.1)	0.5–3380 (79.2)
Peanut					
OFC	Passed	47	1–17	0.18–4.12 (0.85)	0.19–13.2 (1.18)
OFC	Failed	44	1–16	0.14–7.09 (1.35)	0.15–13.6 (1.99)
History positive, not challenged		213	1–18	0.31–2005 (40.4)	0.41–4040 (85.8)

Abbreviations: DBPCFC, double-blind, placebo-controlled food challenge; OFC, open food challenge.

IMMULITE:ImmunoCAP IgE Antibody Ratios for the 3 Food Specificities

From Figures 1 through 3, it is not possible to visually assess the actual magnitude of the differences obtained between the ImmunoCAP and IMMULITE IgE antibody measurements with the different serum samples. For this reason, Figures 4, 5, and 6 display the IMMULITE:ImmunoCAP ratio differences for egg, milk, and peanut specific IgE results, respectively, for the combined groups of patients. The 120 serum samples from egg white sensitized and symptomatic individuals generated a mean IMMULITE:ImmunoCAP ratio of 4.85. In contrast, the 135 serum samples from milk sensitized and symptomatic individuals produced a mean IMMULITE:ImmunoCAP ratio of 2.33. Of the 3 foods, peanut displayed the closest intermethod agreement, with a mean IMMULITE:ImmunoCAP ratio of 1.86. Thus, if a 2-kU/L ImmunoCAP measurement were used in an allergy clinic to determine suitability for entrance into an open peanut challenge, then an

equivalent IMMULITE peanut specific IgE target level would be approximately 4 kU/L.

Relative Median Levels of Food Specific IgE in Food Challenged Patients

Figure 7 presents the IgE antibody concentrations as measured with the ImmunoCAP and IMMULITE systems for those patients who passed or failed an OFC with milk (*left 2 panels*) or peanut (*right 2 panels*). The only significant intermethod difference observed for any of the groups of patients who underwent an OFC was seen in those who failed their peanut challenge ($P = .04$, $n = 34$; median of 1.3 kU/L for ImmunoCAP vs median of 2.0 kU/L for IMMULITE).

DISCUSSION

By 2001, the quantity of egg, milk, peanut, and fish specific IgE antibody in a child's serum as measured by ImmunoCAP

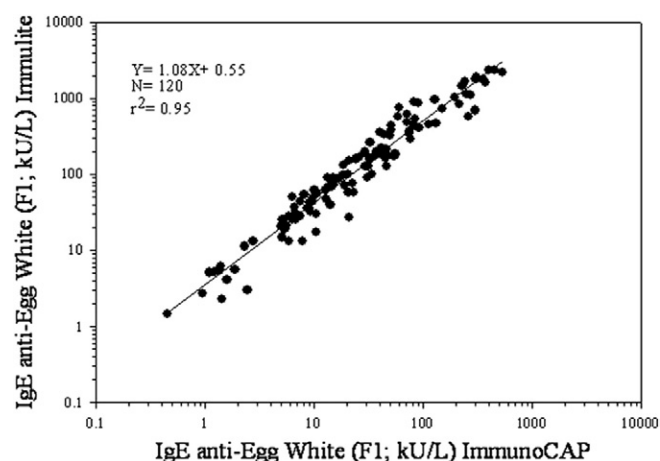


Figure 1. Correlation plot of egg white IgE antibody levels as measured with ImmunoCAP (abscissa) and IMMULITE (ordinate) for 120 serum samples from individuals who were sensitized and had a strong history of egg allergy.

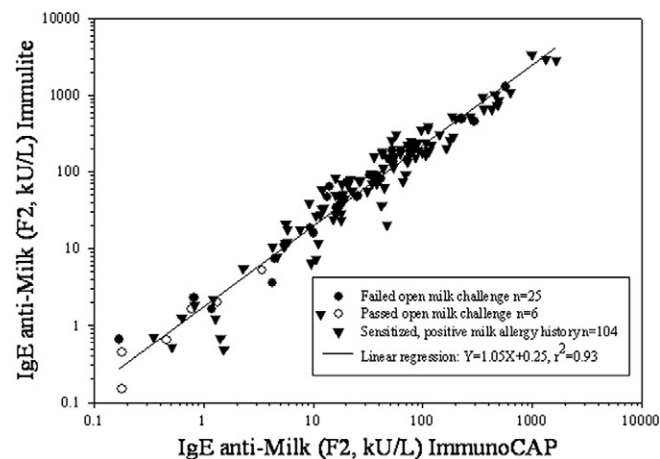


Figure 2. Correlation plot of milk IgE antibody levels as measured with ImmunoCAP (abscissa) and IMMULITE (ordinate) for 135 serum samples from individuals who failed (closed circles, $n = 25$) and passed (open circles; $n = 6$) an open food challenge and individuals who were sensitized and had a strong history of milk allergy (closed triangles, $n = 104$).

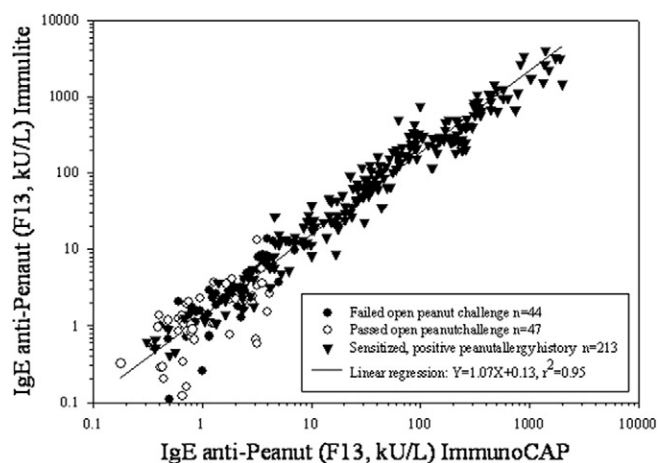


Figure 3. Correlation plot of peanut IgE antibody levels as measured with ImmunoCAP (abscissa) and IMMULITE (ordinate) for 304 serum samples from individuals who failed (closed circles, $n = 44$) and passed (open circles; $n = 47$) an open food challenge and individuals who were sensitized and had a strong history of peanut allergy (closed triangles, $n = 213$).

was viewed by pediatric allergists as a useful marker in the decision to initiate or eliminate a food challenge in the diagnostic workup for food allergy.^{3,4} Following these reports, other investigators performed analogous studies and reported their own predictive values. Because different populations of patients were studied and investigators used different study designs and analytical strategies, these subsequent articles reported different predictive cut points for egg, milk, and peanut.⁵⁻¹⁵ The 2010 National Institute of Allergy and Infectious Disease (NIAID)–sponsored expert panel report on the diagnosis and management of food allergy in the United States¹⁶ summarized this issue by concluding that

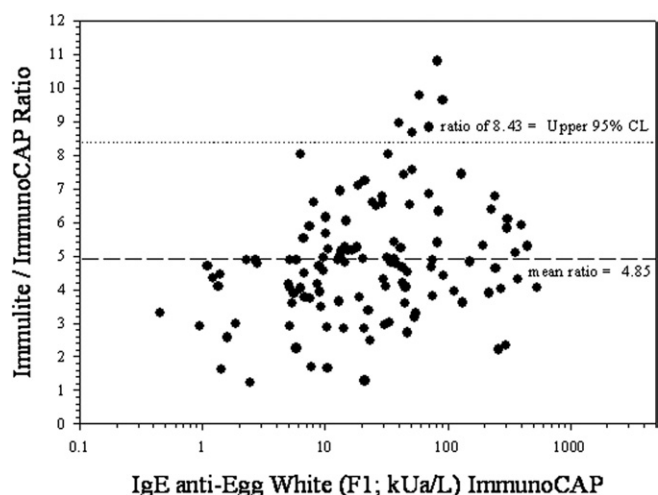


Figure 4. Plot of the IMMULITE:ImmunoCAP ratios for the egg white IgE antibody levels as measured in 120 serum samples from sensitized and symptomatic individuals (mean $\pm$ 1 SD, 4.85 ± 1.79). Ninety-five percent of the study patients had an IMMULITE:ImmunoCAP ratio of 8.43 or less.

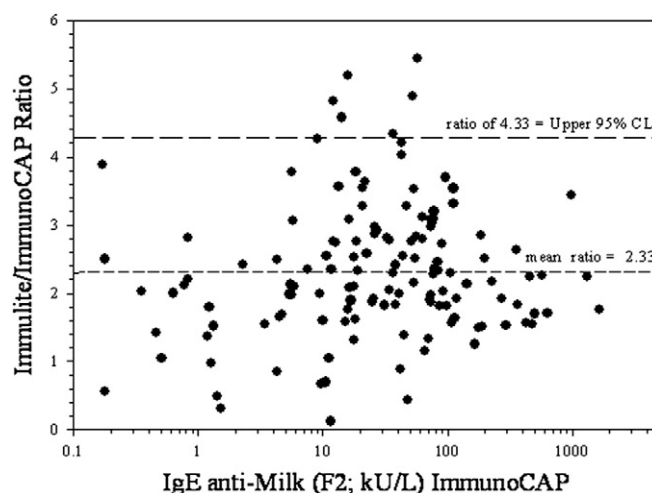


Figure 5. Plot of the IMMULITE:ImmunoCAP ratios for the milk IgE antibody levels as measured in 135 serum samples from sensitized and symptomatic individuals (mean $\pm$ 1 SD, 2.33 ± 1.0). Ninety-five percent of the study patients had an IMMULITE:ImmunoCAP ratio of 4.33 or less.

predictive values are associated with clinical evidence of food allergy. The published predictive values in the literature to date have been generated with the ImmunoCAP system and are then applied judiciously by clinicians, considering the uniqueness of their patient population. Moreover, multiple studies have shown that the other 2 principal IgE antibody assays used in North America (IMMULITE and HyTech-288 [Hycor Biomedical, Garden Grove, California]) report levels of IgE antibody that differ from the ImmunoCAP for egg, milk, and peanut.^{2,17-19} Thus, the predictive values reported in

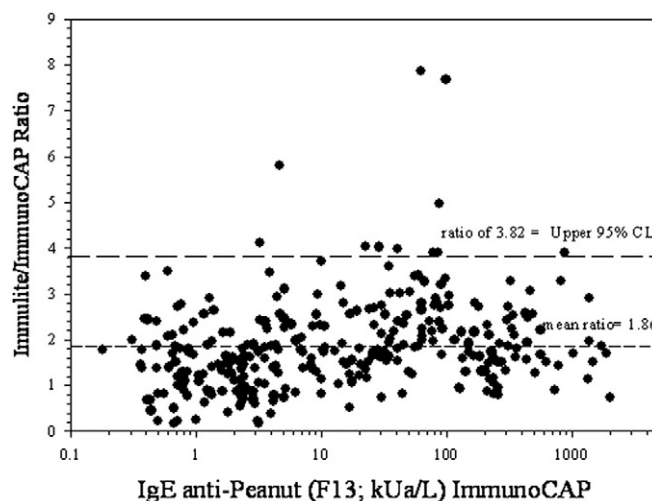


Figure 6. Plot of the IMMULITE:ImmunoCAP ratios for the peanut IgE antibody levels as measured in 304 serum samples from sensitized and symptomatic individuals (mean $\pm$ 1 SD, 1.86 ± 0.98). Ninety-five percent of the study patients had an IMMULITE:ImmunoCAP ratio of 3.82 or less.

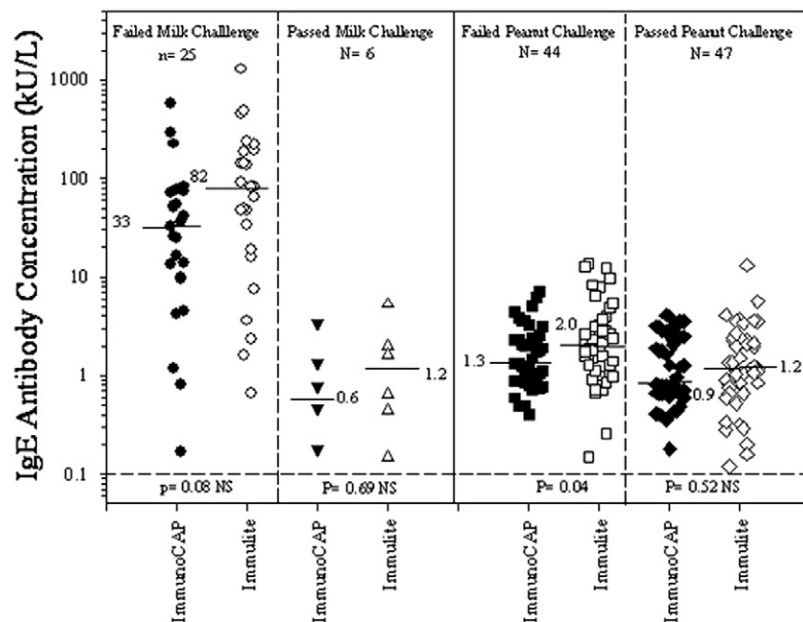


Figure 7. Levels of milk IgE antibody (left 2 panels) and peanut IgE antibody (right 2 panels) as measured by ImmunoCAP and IMMULITE in the serum samples of patients who failed and passed a milk or peanut challenge, respectively. The horizontal lines in each data column indicate the median value of IgE antibody for that group. The only data that displayed a significant intermethod difference (eg, $P = .04$) was in the peanut group that failed their peanut challenge.

the literature have not been considered applicable to data generated with other assay systems.

In this study, we report a systematic intermethod evaluation of egg white, cow's milk, and peanut specific IgE antibody test results with the goal of documenting the relationship between the levels of IgE antibody as measured in the ImmunoCAP and IMMULITE systems. Contrary to suggestions^{17,18} that there may be large differences in reported IgE antibody levels generated with ImmunoCAP and IMMULITE, we found the differences to be definable and modest. IgE antibody levels as measured with ImmunoCAP and IMMULITE were highly correlated for egg, milk, and peanut (Figs 1-3). These data support the conclusions of the 2010 proficiency-based intermethod performance assessment² that both assay methods detect IgE antibody to a comparable degree in serum of symptomatic allergic patients. Examining the variance more closely, mean peanut specific IgE results as measured by IMMULITE differed from ImmunoCAP measurements by 1.86-fold (Fig 6). Statistically, there was no difference in the peanut or milk IgE antibody levels as measured by the 2 assay systems for those who passed and failed an open peanut challenge (Fig 7). Milk specific IgE results as measured by IMMULITE differed from ImmunoCAP measurements by a mean of 2.33-fold (Fig 5). Egg specific IgE levels showed more variance than peanut and milk because levels measured by IMMULITE were a mean of 4.85-fold higher than those measured by ImmunoCAP.

The limitations of this study stem from the difficulty in identifying sufficient serum samples from children who had

undergone an egg challenge because most patients in our clinic begin with a challenge to baked egg. Thus, in this report, we present only intermethod comparative data from children who had a clear history of an allergic reaction after consumption of egg or an egg IgE level that precluded an egg challenge. In addition, this study was not designed to identify the cause(s) of the differences in the levels of IgE antibody as measured by the ImmunoCAP and IMMULITE. Speculation has suggested that these differences may stem from either the heterogeneity of the allergen source used to produce the assay reagents or the method of assay calibration as indicated in the "Methods" section. Because both assays are calibrated against the same WHO IgE reference serum and there does not seem to be a constant systematic bias for all the allergen specificities, heterogeneity of the egg, milk, and peanut extracts, the solid-phase matrix, and the method of immobilization involved in the preparation of the allergen-containing reagent used in each of the assays may be a more plausible explanation for interassay differences. If this is the case, future use of recombinant and native allergen components may largely resolve intermethod differences.

In conclusion, the data in this report suggest that egg, milk, and peanut specific IgE antibody measurements generated by the IMMULITE system most often are higher than those determined by ImmunoCAP, but they can be used with some confidence to make decisions about whether an oral food challenge is needed to confirm symptomatic food allergy. The clinician may choose to translate published ImmunoCAP-based predictive data into comparable IMMULITE levels or generate new predictive criteria based on their own experience with

prospectively collected IMMULITE results. In either case, the clinician must ultimately follow the NIAID guideline's conclusion that IgE antibody measurements generated by any serologic assay method alone are not diagnostic of food allergy.¹⁶ They must be ultimately interpreted within the context of the patient's clinical history.

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