
Correspondence

HYCOR TURBO-MP SPECIFIC IgE ASSAY PERFORMANCE

To the Editor:

We read with great interest the article by Wood et al¹ entitled "Accuracy of IgE Antibody Laboratory Results" and have the following comments in rebuttal of some of their statements and conclusions. The authors of the new study stated that the patient samples tested were randomly selected. However, their further statements indicate that the samples were, in fact, selected based on ImmunoCAP results. It is important to realize that most samples submitted to clinical laboratories test negative for any given allergen. To produce the study sets with the number of positive samples that the investigators tested for peanut and soy, they had to intentionally select for ImmunoCAP-positive samples. This effectively served to exclude samples that were positive by the alternate method(s) and negative for ImmunoCAP. Since no clinical data were presented for the samples chosen, there is no basis for determining which of their samples produced true-positive results. Unfortunately, the investigators did not take this fact into consideration when interpreting their results and came to the mistaken conclusion that the other methods were less sensitive than the ImmunoCAP method.

The authors claimed that the Turbo-MP method failed to detect IgE in several samples that produced positive results by ImmunoCAP. However, the authors used the wrong lower limit of quantitation (LLOQ) for the Turbo-MP method. We suspect that applying the appropriate LLOQ would improve the dichotomous correlation with ImmunoCAP. In any case, it was not appropriate for the authors to state conclusions about the classification of samples by the Turbo-MP method using an LLOQ different from the one in the manufacturer's package insert.

Perhaps a more important point is the lack of clinical relevance of concentrations around the LLOQ of the various methods. It is well recognized that the clinical thresholds of interest to practicing allergists are at considerably higher concentrations than the LLOQ of the assays tested here. Using the clinical thresholds reported by Sampson² for identifying patients with a 95% probability of allergic reaction, the Hycor and Phadia tests segregated the test population in an identical manner.

In a previously published study, Kontis et al³ reported good correlation between Turbo-MP and ImmunoCAP for the quantitative measurement of 6 major food allergens (including soy and peanut, as well as milk, egg, wheat, and fish). In that study, the overall comparison for all allergens combined produced a slope of 0.99 (95% confidence interval, 0.92–

1.03) and correlation coefficient of 0.81 (95% confidence interval, 0.78–0.84) ($n = 457$). The evaluation of individual allergens revealed a slope of 1.02 to 1.58 ($n = 78$) for peanut and a slope of 0.91 to 1.41 ($n = 73$) for soy. The study by Wood et al¹ evaluated the correlation of quantitative measurements of peanut and soy specific IgE, with a cohort size of 60 and 20 patients, respectively. The testing of such small sample sets serves to limit the statistical power of the analysis. Nonetheless, Wood et al reported a slope for the peanut correlation, which compares favorably with the findings of Kontis et al. The fact that Wood et al reported a poor correlation of soy values may well reflect the relative paucity of samples tested ($n = 20$, with only 17 greater than the LLOQ). A balanced evaluation of the data reported by Wood et al actually supports the conclusion advanced in the earlier, more comprehensive study. Both studies indicate that correlation plots show a symmetrical scatter around the line marking ideal correlation.

We question the validity of conclusions the authors made based on testing of the chimeric IgE proteins. The mouse-human chimeric constructs are monoclonal and of undetermined binding affinity. Real-world antibodies generated in the allergic response are polyclonal and have a range of affinities. It is possible that the allergen-specific portion of the synthetic molecule exhibits specificity for some portion of the solid phase used by Phadia and not by the other manufacturers. This theory would explain both the differences between Phadia and Hycor or Diagnostic Products Corporation and the equivalence shown by Phadia between specific and total IgE measurements. Furthermore, our experience with in-house studies has shown that the Hycor birch and dust mite allergen show good quantitative correlation with ImmunoCAP. It is not appropriate to make clinical conclusions based on the results of chimera testing when they are not consistent with results observed in comparisons using human samples and testing of proficiency specimens.

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The above correspondence was referred to the original authors, who provided the following response:

Thank you for the opportunity to respond to the thoughtful comments by Kontis and Nayak regarding our recent publication.¹ It may be helpful to clarify the objective of our study, which was simply to determine whether different IgE antibody test technologies, which are in common use in commercial laboratories, provide similar quantitative serologic results. If differences in these technologies exist, this information is clinically important, since the IgE antibody levels that have been established using ImmunoCAP technology are routinely used for clinical decision making, whereas the other technologies have not undergone similar study.²⁻⁹ The potential for confusion is compounded by the fact that different commercial laboratories report allergen specific IgE levels in the same units, often without any information regarding the specific assay that was used to determine these levels.

The initial objection of Kontis and Nayak relates to our choice of serum samples. Since our study only focused on serologic patterns, there was no attempt to identify clinical reactivity of individuals from which the samples were derived, although we agree that such a study would be useful in the future. ImmunoCAP was chosen as the reference system to which the other 2 methods were compared, since it has been validated in numerous peer-reviewed publications from around the world.¹⁰

Kontis and Nayak also correctly point out that the different methods have different lower limits of detection. However, since these test systems report results in the same units, mutual cutoffs were used for the qualitative comparisons. From this analysis, we came to the conclusion that some differences exist among the 3 test methods and that Turbo MP differed most. However, to be completely fair, we also presented results using the lower limit of quantitation recommended by the manufacturer and found that the Turbo MP still failed to detect peanut IgE in 7 of 55 samples positive by ImmunoCAP at the same cutoff.

Kontis and Nayak also suggest that it may be more important that the methods compared well at higher antibody levels used to identify patients with a higher probability of clinical reactivity.⁷⁻⁹ This approach suggests greater agreement among the systems, although the high degree of variability between the Turbo MP results and those obtained with the 2 other methods is still a concern. In addition, we and others have shown in multiple studies that clinical decisions are also critical at the lower end of detection, especially to determine if a food allergy may have been outgrown, so the disparities at lower specific IgE levels among the test methods are clearly a concern in the management of patients.^{2-7,11}

Kontis and Nayak state that they have demonstrated good correlation between ImmunoCAP and Turbo MP in a previous publication¹² and that our results may simply reflect an

inadequate sample size. Although we agree that a larger sample size is always preferable in such an analysis, on statistical grounds it is unlikely that a larger sample size would have changed the outcomes of our comparisons or our conclusions. Further, from both clinical and statistical standpoints, we cannot agree with their assertion that their publication demonstrated reliable correlations among the methods, even with a larger sample size. For example, in their publication the slope for peanut was 1.37 with a confidence interval of 1.02 to 1.58, which represents a significant deviation from identity and is also inconsistent with the confidence interval of 0.90 to 1.35 incorrectly stated in their letter.

Finally, with regard to our analysis using chimeric antibodies, we agree that such antibodies are of restricted antibody affinity. However, such antibodies usually have a substantial affinity and should be detectable in a reproducible manner in a high-quality serologic system. Further, the suggestion that the specificities of the chimeric antibodies are only directed to the solid phase in the ImmunoCAP is inconsistent with published data.^{13,14} The commercially available anti-Der p 2 chimeric antibody has also been successfully applied as an allergen (Der p 2)-specific calibrator in an enzyme-linked immunosorbent assay system.³ The theory that chimeric antibodies are immunologically measurable as IgE molecules was verified in our study when they were determined as total IgE by all systems. However, when they were determined as allergen specific IgE antibodies, only the ImmunoCAP system showed the expected results. Since our study focused on the serologic performance of the different technologies used, our conclusion that the different test technologies do not provide similar IgE antibody information is justified.

In conclusion, this an area of potentially great clinical significance that clearly deserves further study. Additional studies with larger numbers of samples that include information on clinical reactivity will be needed to more completely clarify the concerns raised by Kontis and Nayak, as well as many other unanswered questions.

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ARE WE FOLLOWING PRACTICE PARAMETER GUIDELINES ON ALLERGEN IMMUNOTHERAPY?

To the Editor:

With the release of the new allergen immunotherapy practice parameters, allergy practices should evaluate if they are following practice parameter recommendations. I have found that some allergists continue the same prescription patterns as when they started practicing allergy. Generally, if a junior associate joins the practice, he or she continues the prescription patterns that were in place before he or she joined. We should be vigilant in following the current guidelines that our professional societies have set forth for us.

One concern is the extreme variations in maintenance dosing. These variations include 10-fold (1:10), 100-fold (1:100), and even 1,000-fold (1:1,000) dilutions and aiming for those dilutions as the maintenance vial (nomenclature based on suggested nomenclature from the allergen immunotherapy practice parameter).¹ These dilutions likely contain significantly lower doses than the practice parameter's probable effective dose range. For example, if a 5-mL maintenance vial contains 2 mL of *Dermatophagoides pteronyssinus*, the maintenance dose (assuming 0.5 mL was used as the maintenance injection) would contain 2,000 AU of *D pteronyssinus*. If a 100-fold dilution is now used for the maintenance dose and assuming the same numbers as above, the patient is only receiving 20 AU of *D pteronyssinus*, which is significantly lower than recommended doses (500 to 2,000

AU).¹ Patients may feel better while receiving low-dose immunotherapy, but it would be difficult to evaluate the improvement due to the actual treatment vs a placebo effect. Some patients may only tolerate lower doses because of systemic or local reactions, but treatment should initially be aimed at targeting practice parameter dosing.

Another issue we should consider in our prescription patterns is the glycerin content in the maintenance vials. Glycerin may lead to significant painful reactions that prevent patients from going to higher doses in their immunotherapy. Some of the standardized allergens (such as cat and dust mite) come in 50% glycerin. We have the option of getting glycerinated or unglycerinated extracts in regard to the tree and weed extracts. Some choose the 1:20 (wt/vol) extracts for their tree and weed extracts because of the cheaper cost and the later expiration date. This may make it difficult to keep the maintenance vial less than 30% glycerinated unless using significant amounts of diluent. If in a 5-mL maintenance vial 4 mL is glycerin-containing (1:20 wt/vol) tree and weed extracts and the rest diluent, already the glycerin content is 40%. My suggestion is to consider using 1:10 (wt/vol) tree and weed extracts to keep the glycerin content below 30%. Another suggestion is to consider contacting the doctors from the extract manufacturing company to help review your practice's immunotherapy prescription patterns.

Immunotherapy, like other areas of medicine, is an art based on science. There are many nuances and variations based on style, but the fundamental practice should still be based on science and the practice parameters recommendations.

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ACCURACY OF IgE ANTIBODY LABORATORY RESULTS

To the Editor:

Wood et al¹ attempted to suggest that the quantitative disparity in different manufacturers' specific IgE (sIgE) results raises the question of which manufacturer is accurately measuring IgE. They conclude that ImmunoCAP is the only assay that accurately measures sIgE because it shows a 1-to-1 correlation between sIgE and total IgE results from the measurement of chimeric antibodies. This conclusion ignores some of the key, basic tenets of immunology, particularly antibody affinity, antibody avidity, and how these affect sIgE

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results. The role of affinity in sIgE measurements has been discussed and reported in various publications.²⁻⁴

It is well established that sIgE assays give different quantitative measurements on the same sample owing to differences in allergens, antibody affinity and avidity, matrix (liquid vs solid), and assay formats. Thus, the quantitative differences between assay results illustrated in the linear regression and Bland-Altman plots (Figs 1 and 2 in the study by Wood et al¹) are to be expected irrespective of which manufacturer's assay was used as the reference method. In addition, the absolute quantitation of sIgE antibodies is complicated owing to the heterogeneity of the allergens. In patient samples, the presence of potentially interfering antibodies of other isotypes directed to the same allergenic epitopes and the presence of cross-reactive determinants also affect sIgE results. Furthermore, Ollert et al⁵ demonstrated that although results for ImmunoCAP and Immulite were quantitatively different, Immulite was equivalent to ImmunoCAP for accurate diagnosis of allergic disease and correlated better with skin test results.

Standardization to IgE units has been adopted by manufacturers as a means of heterologous standardization for sIgE assays. The authors' premise is that using an accurate method, the total IgE result should equal the sIgE result when measuring a chimeric antibody. However, owing to the differences in the antibodies, allergens, and assay formats used in sIgE and total IgE assays, one would not expect a 1-to-1 correlation between sIgE and total IgE assay results. Rather, sIgE assays may overrecover or underrecover relative to total IgE as a result of the different affinities of the allergen recognizing the IgE antibody and the anti-IgE antibody used

for detection, although a 1-to-1 correlation may occur if the assays are designed for that purpose. Thus, although the chimeric antibody is an interesting test system, a 1-to-1 correlation between sIgE and total IgE measurements does not prove that the assay is providing the true result. The model described by Wood et al¹ is relevant only if one assumes that all patient antibodies have the same avidity as the chimeric antibody for an allergen. The disparity in results reported by Wood et al is likely due to differences in assay formats, antibody affinities, and allergen extracts.

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