

Quantitation of Specific IgE by the Hycor Ultra-Sensitive Specific IgE EIA System

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A number of methods for diagnosing atopic responses are available to the clinician, including skin prick testing, intradermal dilution testing, clinical history, oral challenge, and *in vitro* measurement of serum specific and/or total IgE.^{1,2} *In vitro* measurement of allergen-specific serum IgE can be used to help determine atopic response and guide clinicians in advising their patients in allergy management and treatment.^{3,4} Serum assays for specific IgE are available from a number of suppliers, and the comparability of these products in terms of linearity and assay response is of interest to clinicians who are diagnosing and designing the treatment of allergy patients. We have measured the dilution linearity and limits of detection and quantitation of the Hycor Ultra-Sensitive Specific IgE EIA System, which is calibrated to the international WHO IgE standard (IRP 75/702). We directly compared the performance of the Hycor allergy system to that of Phadia ImmunoCAP FEIA[®], (“Phadia ImmunoCAP”). Assay results on the two systems were plotted for approximately fifty patient samples for each of a group of common allergens. Close comparability is seen in all cases in the response of these assays to patients’ specific IgE (Fig. 2-13). Phadia ImmunoCAP results were obtained by IBT Reference Laboratory, Lenexa, Kansas.

Dilution Linearity of the Hycor Ultra-sensitive Specific IgE EIA system

Serial dilutions of samples from patients with sensitivities to eight common allergens were performed across the assay’s linear range, from > 2 kU/L to < 0.1 kU/L, and assayed on the Hycor Ultrasensitive Specific IgE EIA System, in order to determine linearity. For each allergen, the best-fit line (linear least squares regression) had correlation coefficient (r^2) greater than 0.99.

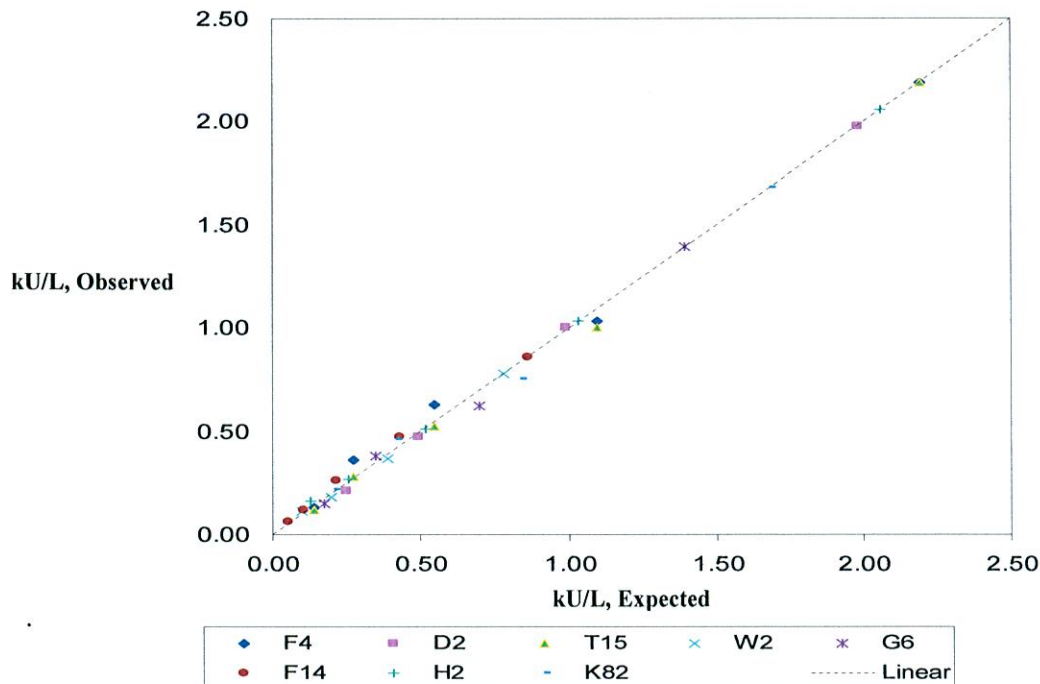


Figure 1. Dilution linearity for eight allergens

Limit of Detection and Limit of Quantitation of the HYPOR Ultrasensitive Specific IgE EIA system

The Clinical and Laboratory Standards Institute (CLSI) documents I/LA20-A2⁵ and EP-17A⁶ provide a methodology for determining Limit of Detection in specific IgE assays: assuming an equivalent distribution between the zero calibrator and low positives, the LoD is set the point at which there is a 5% or smaller chance of the measurement on a sample with that concentration falling in the 95% confidence interval of the zero as determined on multiple occasions over multiple days. Using this methodology, the Limit of Detection of the HYPOR Ultrasensitive Specific IgE EIA System was determined to be 0.04 kU/L, allowing confidence in detection of very low specific IgE levels.

Similarly, CLSI document EP-17A provides a methodology for determining Limit of Quantitation: the lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness, under stated experimental conditions. Using methodology detailed in EP-17A, the limit of quantitation of the HYPOR Ultrasensitive Specific IgE EIA System was determined to be 0.07 kU/L, allowing excellent sensitivity.

Correlation of Phadia ImmunoCAP and HYPOR results for Common Allergens

Plots correlating results from the HYPOR and Phadia assays for common allergens (Fig. 2-13) cluster around the line of identity. Considerable variability is seen in individual patient responses, and this is not unexpected. HYPOR technology assesses the patient's response to a wide variety of protein components, and only employs those found naturally in the allergen source. It would be possible for HYPOR to obtain artificially elevated results by 'spiking' our allergen preparations with genetically modified material, but we feel the most accurate results are obtained by challenging the patient's serum with naturally-occurring material that originates with the actual allergen source.

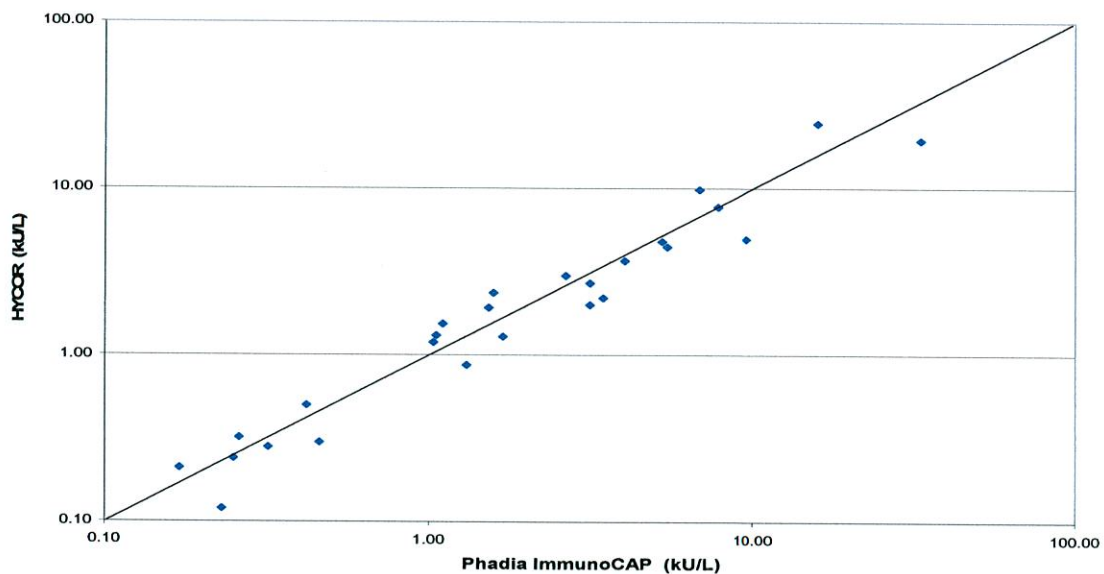


Figure 1. W1 (Common Ragweed)

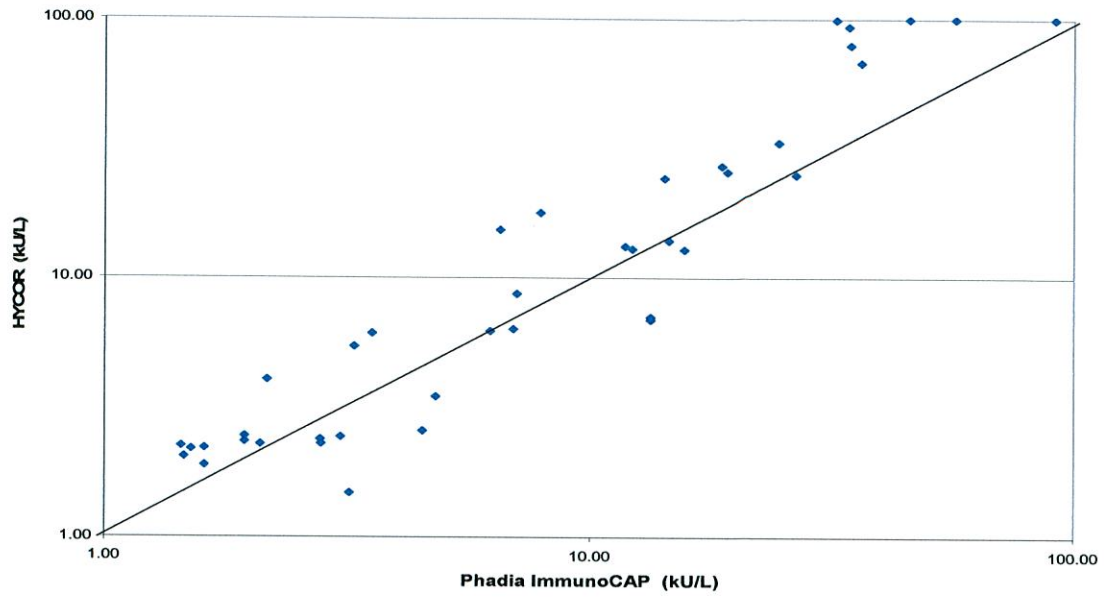


Figure 2. F75 (Egg Yolk)

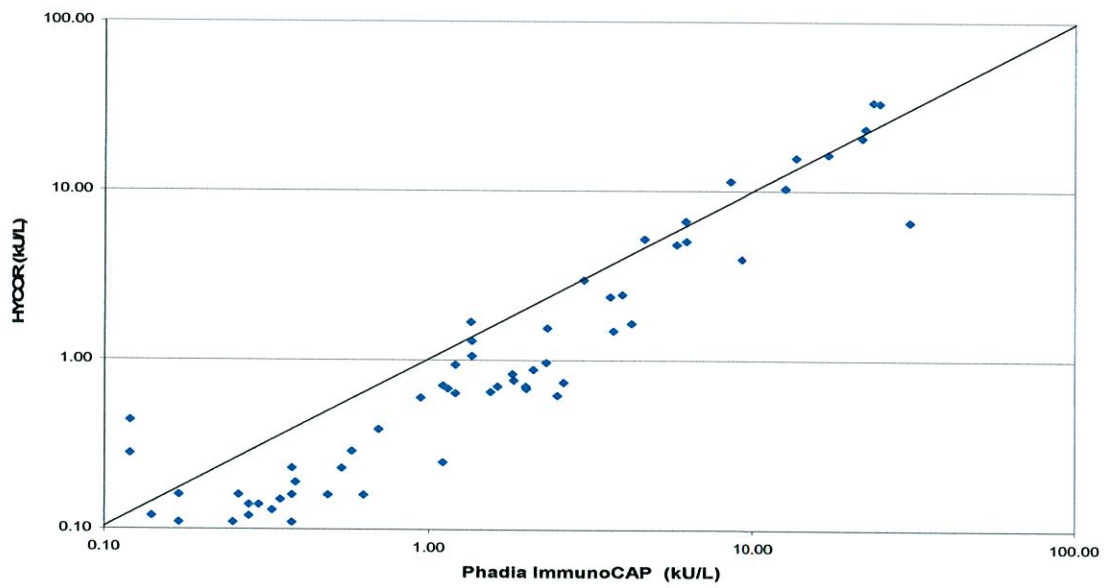


Figure 3. F1 (Egg White)

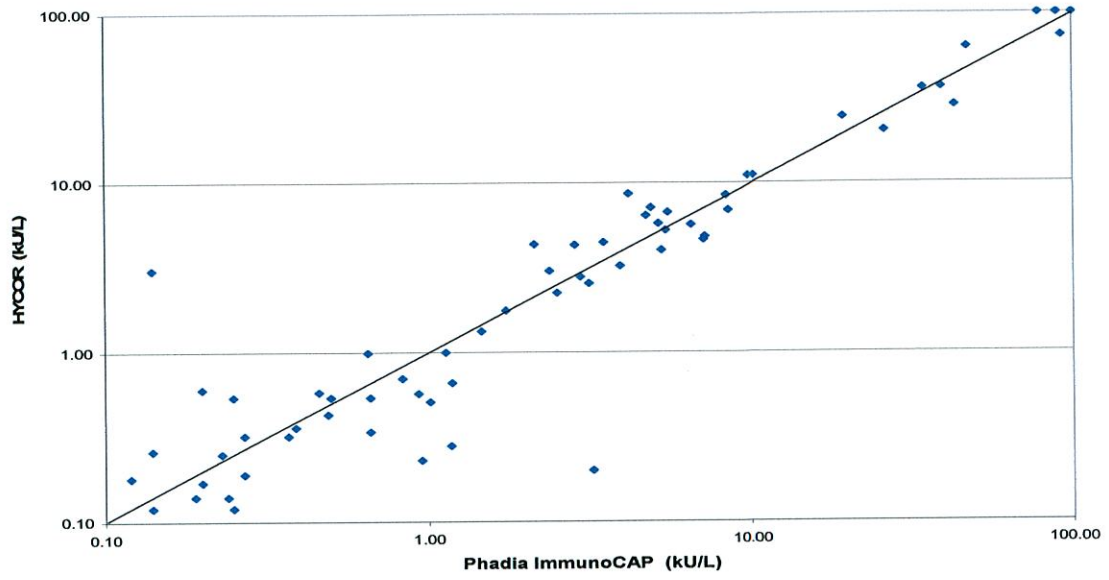


Figure 5. D2 (House Dust Mite, *D. farinae*)

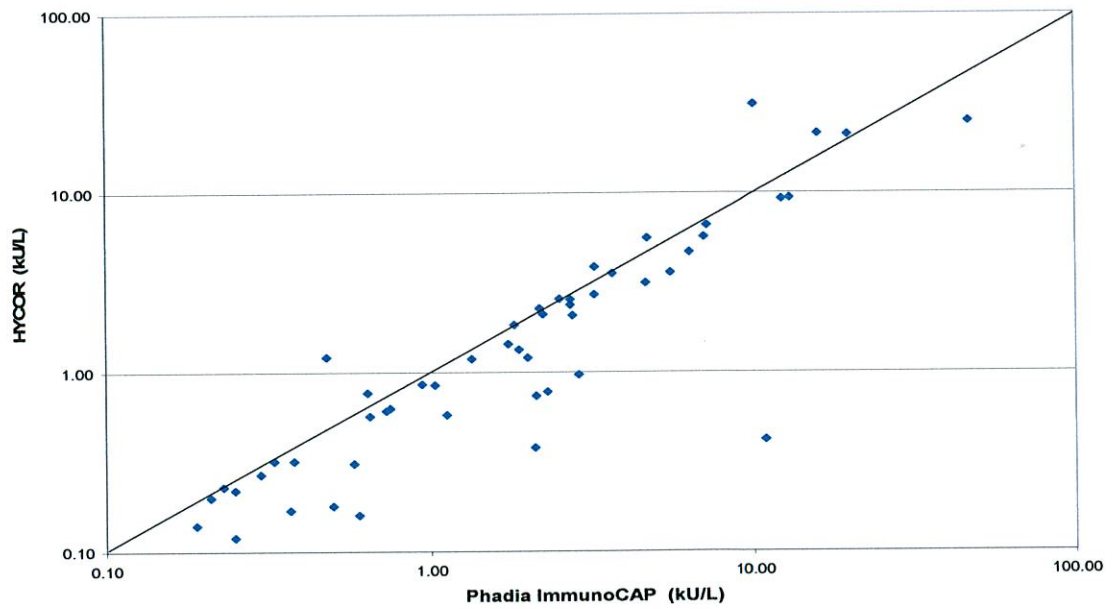


Figure 6. E1 (Cat Hair / Dander)

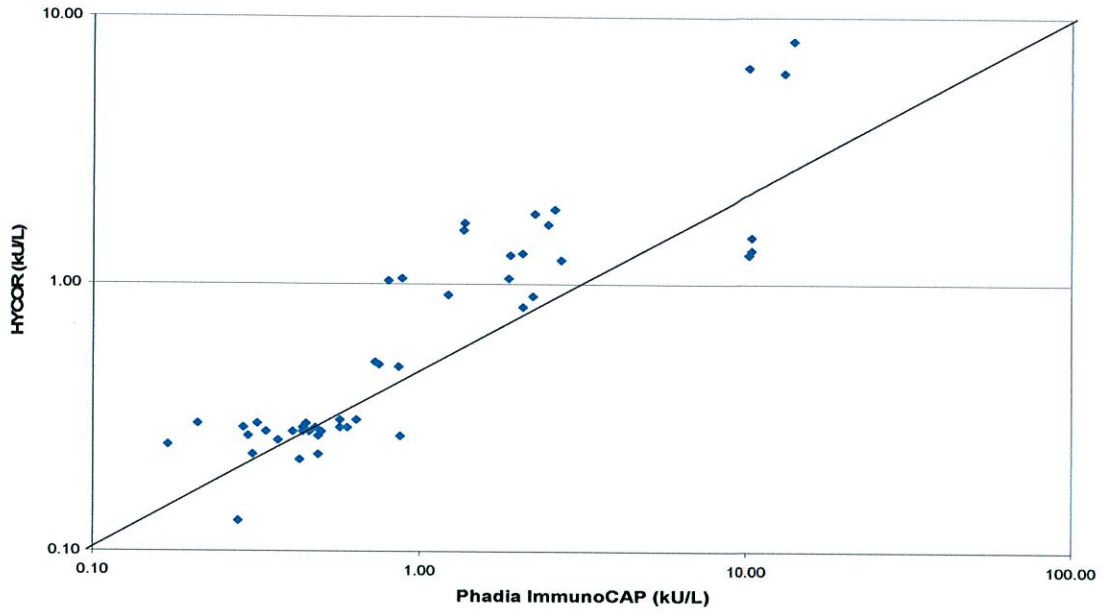


Figure 9. I3 (Common Wasp / Yellowjacket)

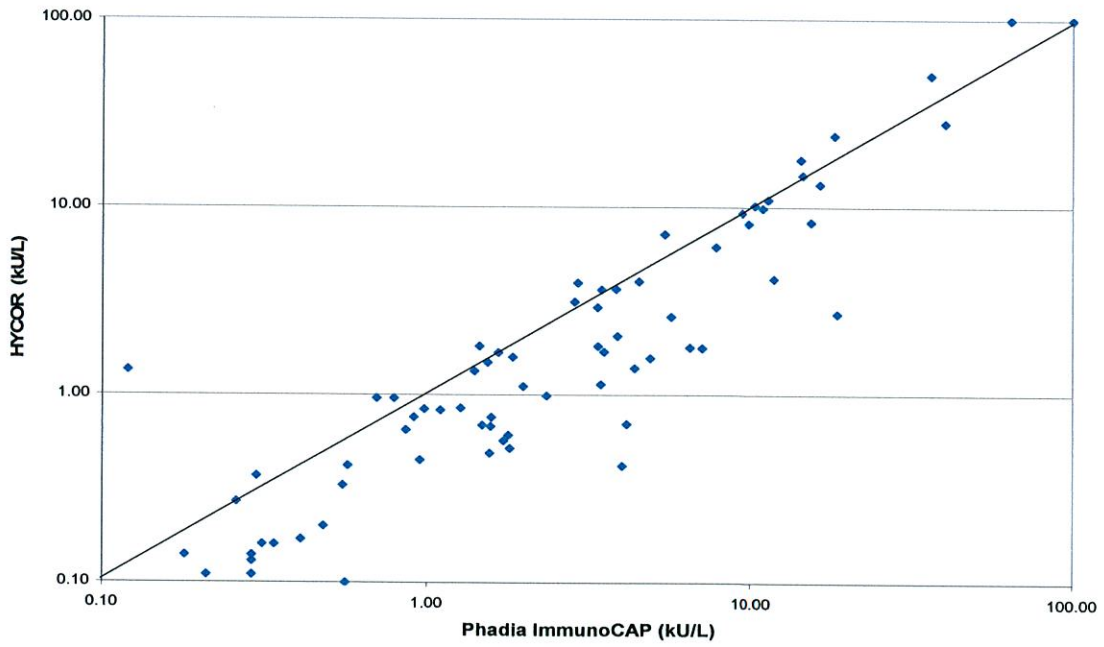


Figure 8. F13 (Peanut)

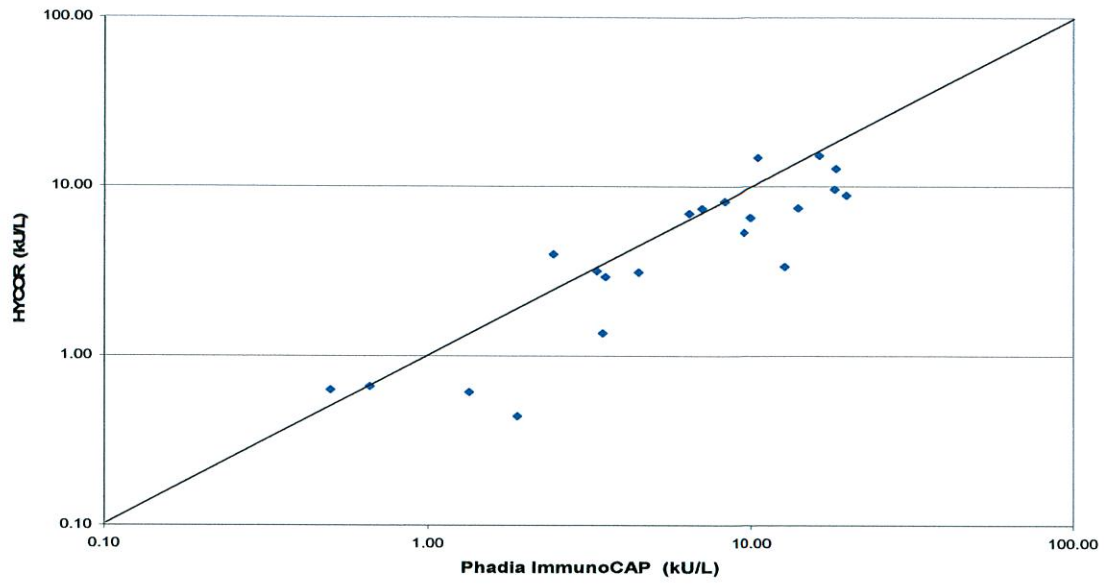


Figure 7. F3 (Cod)

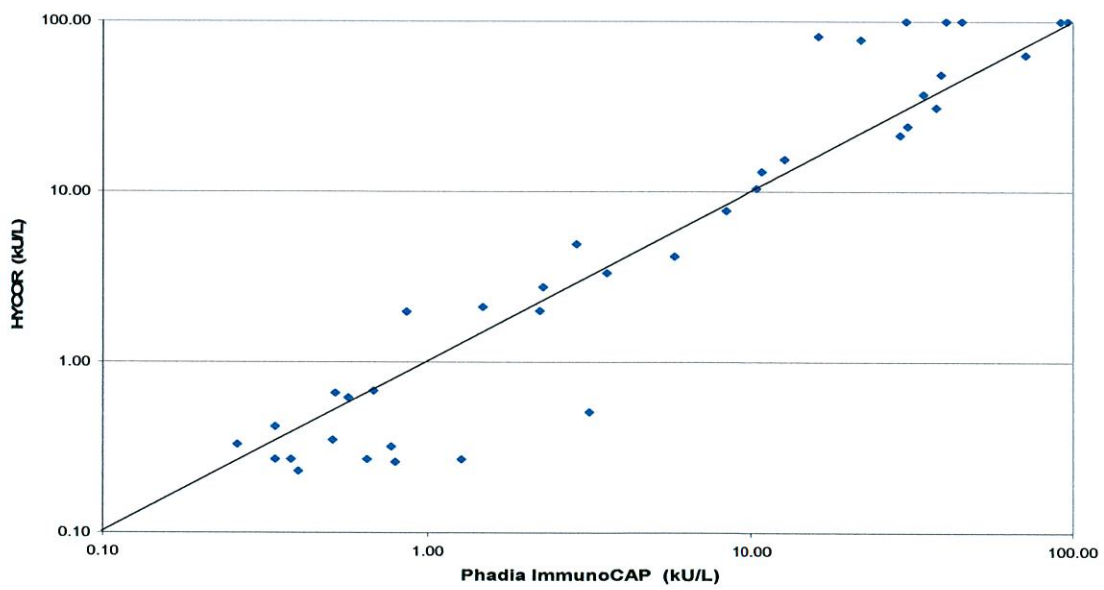


Figure 10. T7 (White Oak)

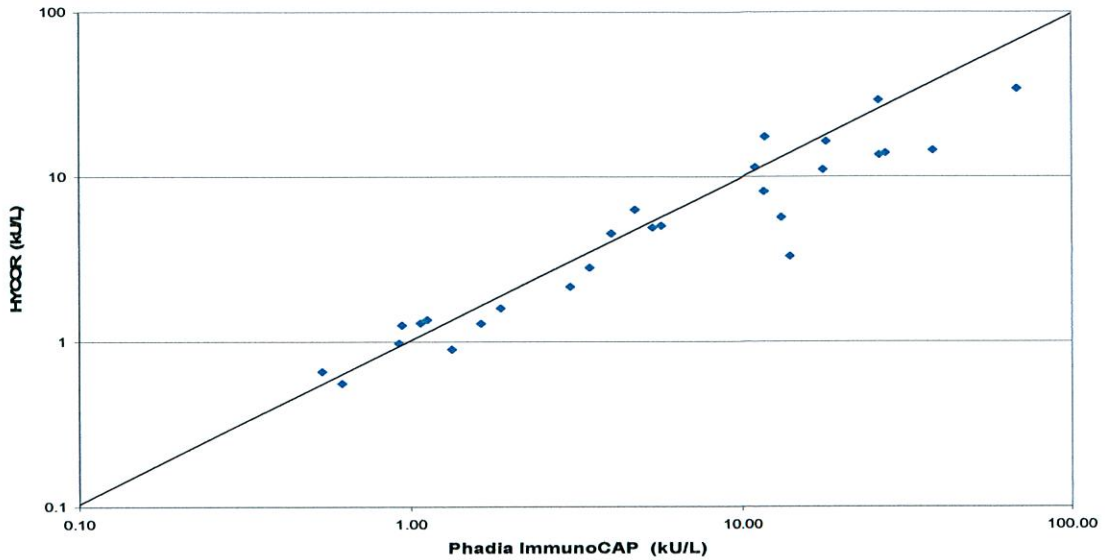


Figure 11. M6 (*Alternaria tenuis*)

Quantitation of low-end positives

In terms of analytic sensitivity, there is a difference between HYCOR and Phadia ImmunoCAP results with certain sample types. Table 1 (below) compares results for a group of patients with very low response to Yellow Jacket venom as an example. HYCOR's assay is capable of quantitating results below 0.1 kU/L and thus provides quantitative results for these samples, most of which fall in the equivocal or Class I range.

HYCOR ID	CAP	HYCOR
I3-007	<0.10	0.02
I3-008	<0.10	0.05
I3-021	<0.10	0.11
I3-024	<0.10	<0.01
I3-025	<0.10	<0.01
I3-057	<0.10	0.06
I3-061	<0.10	0.06
I3-064	<0.10	<0.01
I3-066	<0.10	0.09
I3-068	<0.10	0.07

Table 1. Phadia ImmunoCAP ("CAP") and HYCOR results for a selection of low positive, equivocal, and negative samples specific for allergen I3, Common Wasp (Yellowjacket).

Phadia ImmunoCAP does not quantitate in this range and reports all the samples as negative. We do not consider this to be discordance, but rather a difference created by the different low-end quantitation design for the two assays. In the example shown in Table 1, HYCOR's higher sensitivity created an apparent discordance in results only in one case: Patient I3-021 is reported as 0.11 kU/L, positive, whereas Phadia ImmunoCAP reports it as less than 0.10 kU/L, which Phadia ImmunoCAP considers negative. Since Phadia does not quantitate below 0.10 kU/L, samples in that region are not included in quantitative comparison.

International Units and Class Scores

Many clinicians use Class Scores in evaluating their patients.⁷ In the Modified Class Scoring system (MCS), each of the six classes encompass a broad range of specific IgE mass (reported in kU/L), recognizing that the mass may not be strongly correlated with strength of allergic response. For example, the difference between a class score of 0, 1, or equivocal may not be clinically significant, and asymptomatic patients with class scores of 2 and higher are not uncommon. Thus a patient may have a specific IgE response that varies over an order of magnitude in kU/L without detectable difference in physiologic response. Each clinician must determine the best measure to use to aid in diagnosis of allergy.

Summary

The HYCOR Ultrasensitive Specific IgE EIA System provides an automated method for determining allergen-specific IgE in serum. The assay demonstrates dilution linearity, industry-leading limits of detection and quantitation, and gives results comparable to those seen with Phadia ImmunoCAP. Patient to patient differences are not generally greater than one 'class', and we suspect such cases are created by different approaches to antigen presentation by the two companies. Only in the case of very low positives do we find a difference in clinical sensitivity between the two methods and this is due more to HYCOR's quantitation ability in this area.

Determination of specific IgE in serum by this system provides excellent sensitivity at low IgE levels. Such determination can provide additional information towards diagnosis and treatment planning for allergy patients.

References

- 1) Kay AB, Barata L, Meng Q, Durham SR, Ying S. 1997. *Int. Arch. Allergy Immunol.* 113, 196-199.
- 2) Bousquet J, Lockey R, Malling HJ 1998 *J. Allergy Clin. Immunol.* 102, 558-562.
- 3) Ceska M and Lundquisk U. 1972. *Immunochemistry* 9, 1021-1025.
- 4) Johansson SG, Berlund A, Kjellman N, 1976. *Clinical Allergy* 5, 91-98.
- 5) CLSI. *Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline* CLSI document I/LA20-A2. CLSI, 940 West Valley Rd, Suite 1400, Wayne, PA 19087.
- 6) CLSI. *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* CLSI document EP-17A. CLSI, 940 West Valley Rd, Suite 1400, Wayne, PA 19087.
- 7) Nalebuff DJ, Fadal RG, and Ali M. The study of IgE in the diagnosis of allergic disorders in an otolaryngology practice. 1978. *Otolaryngol Head Neck Surg* 87, 351-358.