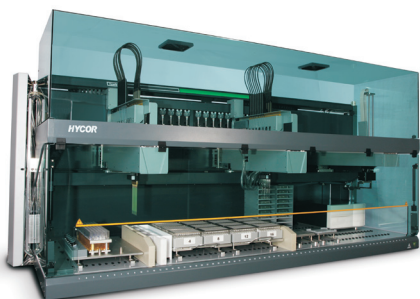


# HYCOR Ultra-Sensitive EIA System for Allergen-Specific IgE

## Quantitation of Specific IgE by the Hycor Ultra-Sensitive 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.<sup>1,2</sup> *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.<sup>3,4</sup> 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 EIA System for Allergen-Specific IgE, 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® (FEIA). 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. 2–11). FEIA results were measured by IBT Reference Laboratory, Lenexa, Kansas.

### Dilution Linearity of the Hycor Ultra-Sensitive EIA System for Allergen-Specific IgE

Serial dilutions of samples from patients with sensitivities to eight common allergens were performed from  $> 2$  kU/L to  $< 0.1$  kU/L, and assayed on the Hycor Ultra-Sensitive 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.

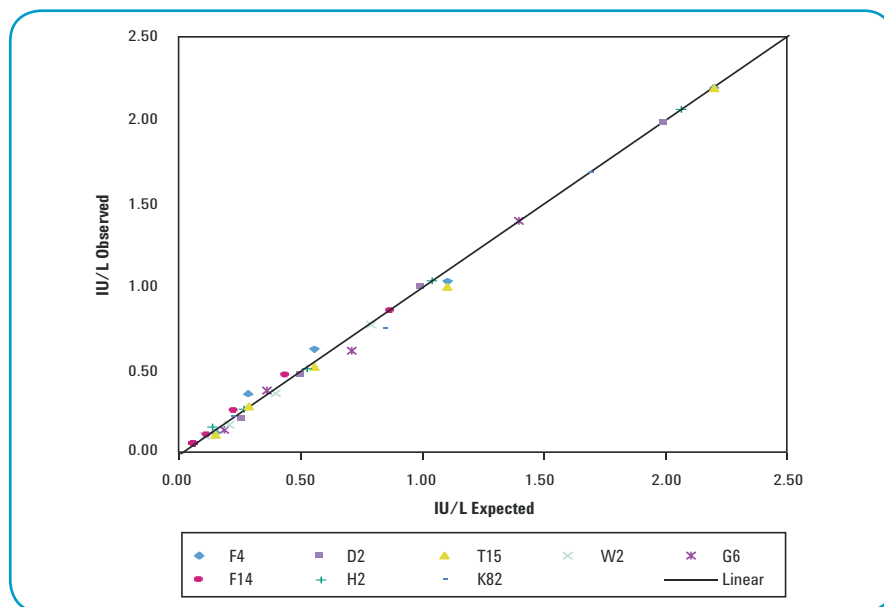


Figure 1. Dilution linearity for eight products, including food, plants, and household allergens



**Limit of Detection and Limit of Quantitation of the HYCOR Ultra-Sensitive EIA System for Allergen-Specific IgE**

The Clinical and Laboratory Standards Institute (CLSI) documents I/LA20-A2<sup>5</sup> and EP-17A<sup>6</sup> provide a methodology for determining Limit of Detection (LoD) in specific IgE assays: assuming an equivalent distribution between the zero calibrator and low positives, the LoD is set at 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 LoD of the HYCOR Ultra-Sensitive 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 HYCOR Ultra-Sensitive EIA System was determined to be 0.07 kU/L, allowing excellent sensitivity.

**Correlation of FEIA and HYCOR Ultra-Sensitive EIA System Results for Common Allergens**

Plots correlating results from the two assays for common allergens (Fig. 2-11) cluster around the line of identity. Considerable variability is seen in individual patient responses, and this is not unexpected. The HYCOR Ultra-Sensitive EIA System 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 the HYCOR Ultra-Sensitive EIA System 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.

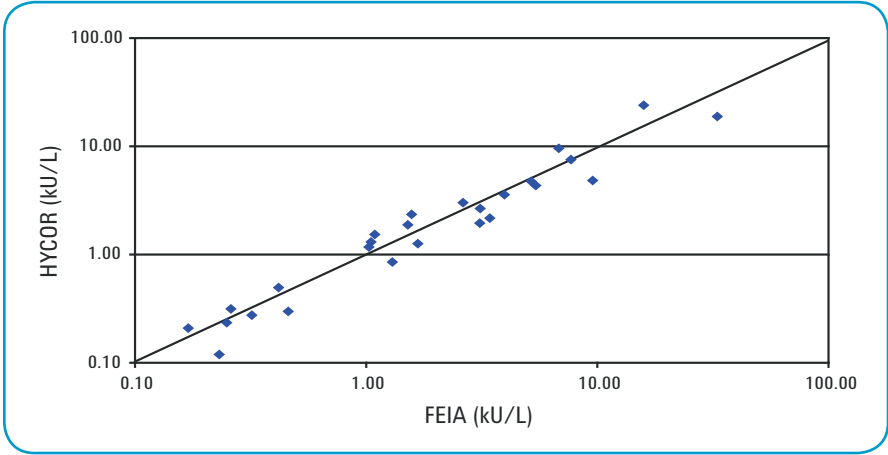


Figure 2. W1 (Common Ragweed)

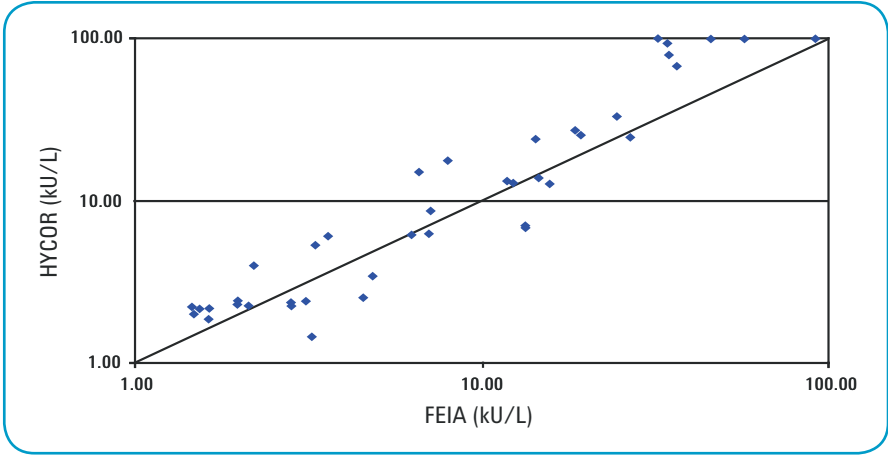


Figure 3. F75 (Egg Yolk)

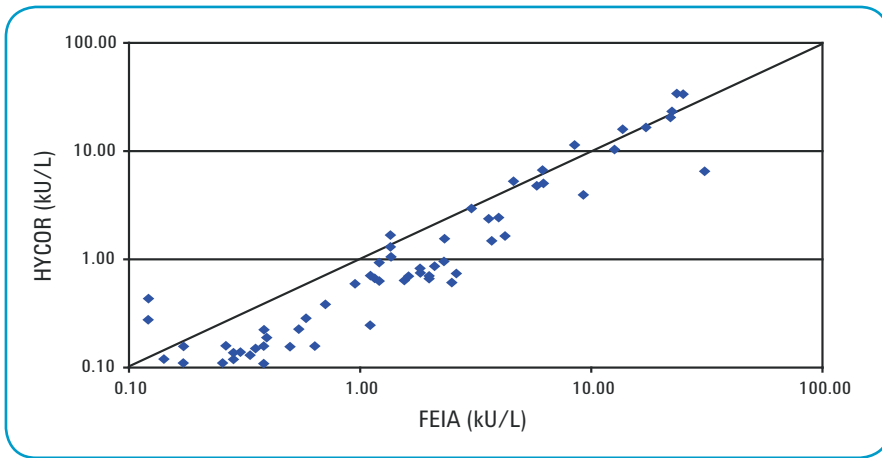


Figure 4. F1 (Egg White)

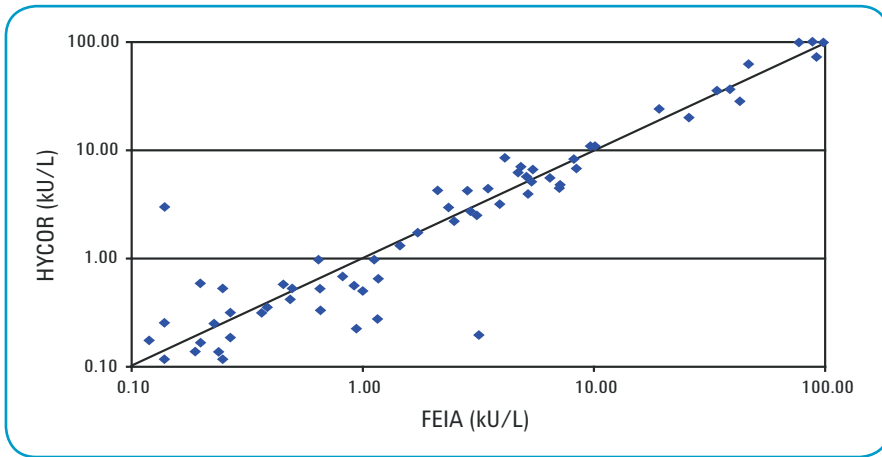


Figure 5. D2 (House Dust Mite, *D. farinae*)

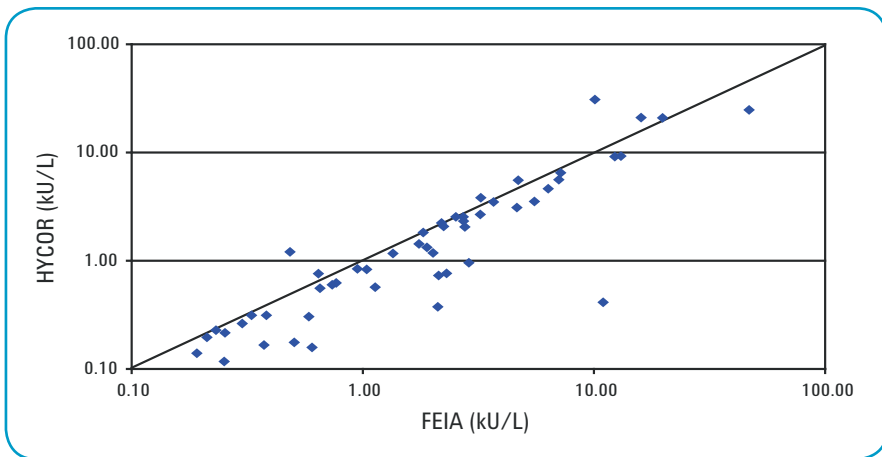


Figure 6. E1 (Cat Hair/Dander)

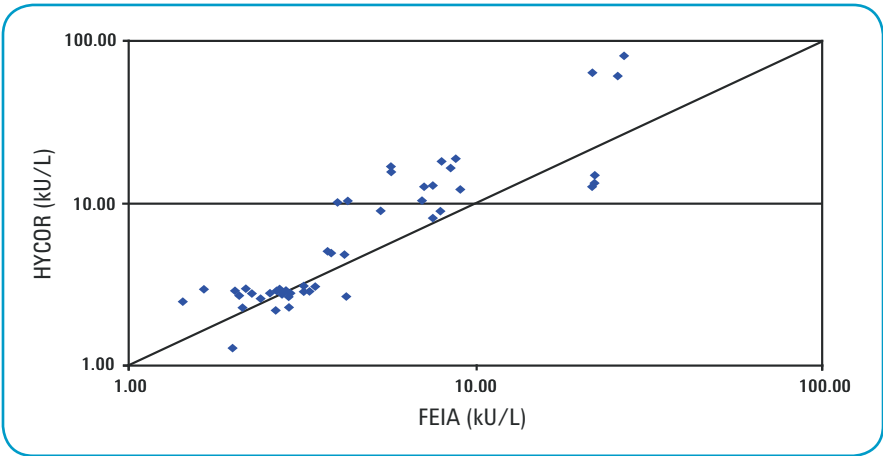


Figure 7. I3 (Common Wasp/Yellow Jacket)

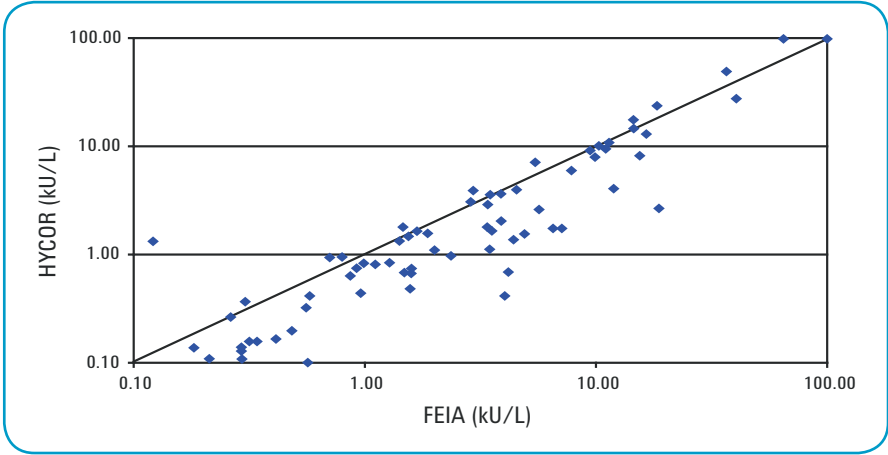


Figure 8. F13 (Peanut)

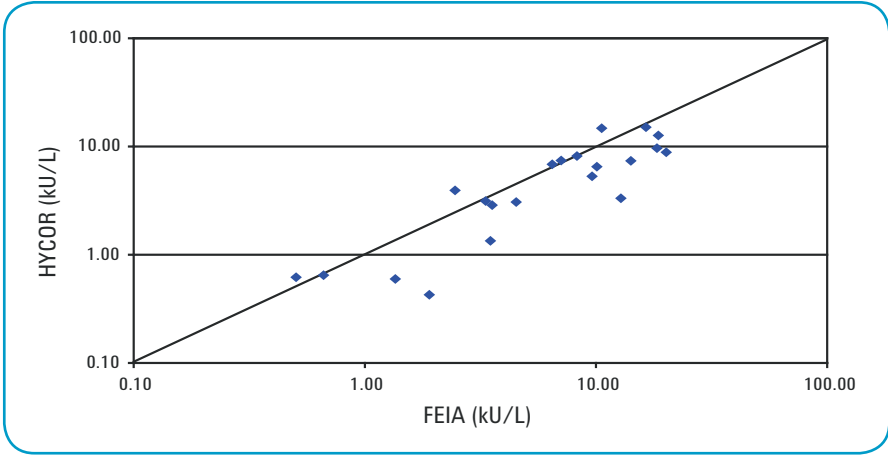


Figure 9. F3 (Cod)

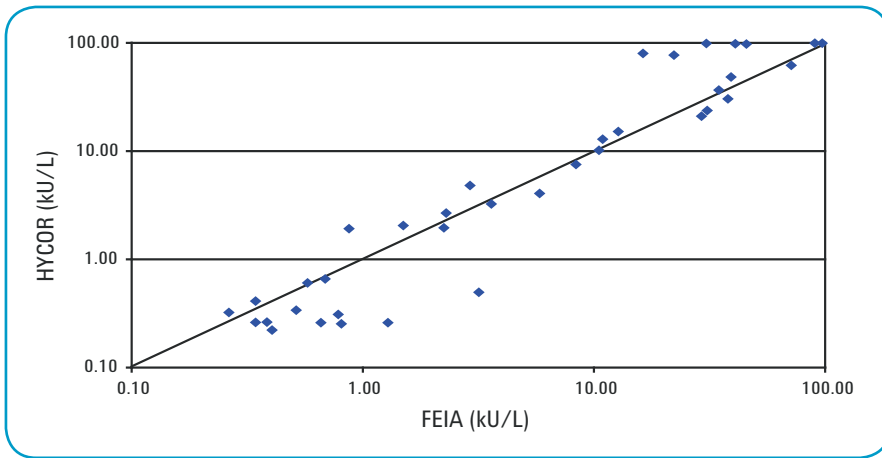


Figure 10. T7 (White Oak)

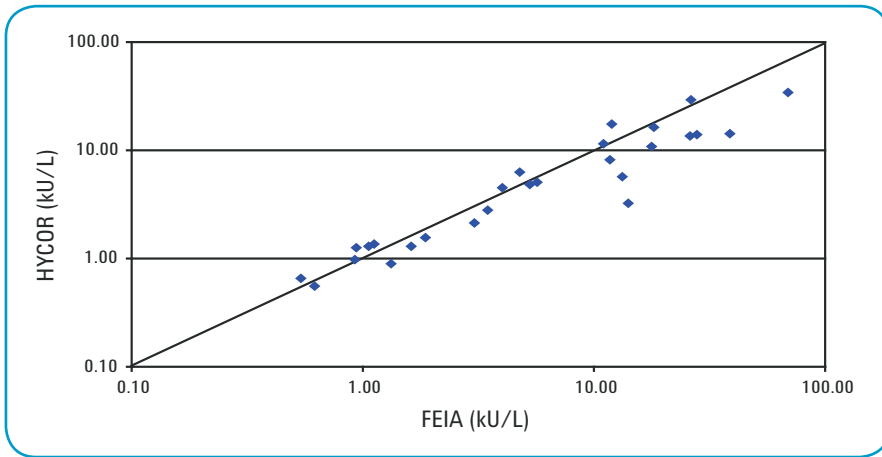


Figure 11. M6 (Alternaria tenuis)

**Quantitation of Low-End Positives**

Reported results are somewhat different for very low samples. Table 1 (below) compares results for a group of patients with very low response to common wasp (yellow jacket) venom as an example. The HYCOR Ultra-Sensitive EIA System 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	FEIA (kU/L)	HYCOR (kU/L)
I3-007	<0.10	<0.04
I3-008	<0.10	0.05
I3-021	<0.10	0.11
I3-024	<0.10	<0.04
I3-025	<0.10	<0.04
I3-057	<0.10	0.06
I3-061	<0.10	0.06
I3-064	<0.10	<0.04
I3-066	<0.10	0.09
I3-068	<0.10	0.07

Table 1. FEIA and HYCOR Ultra-Sensitive EIA System results for a selection of low positive, equivocal, and negative samples specific for allergen I3, common wasp (yellow jacket).

FEIA results reported by the reference laboratory do not quantitate in this range and report all the samples as negative. We do not consider this to be discordance, but rather a difference created by the different low-end report format. In the example shown in Table 1, there is an apparent discordance in results only in one case: Patient I3-021 is reported as 0.11 kU/L, positive, whereas the reference laboratory reported the FEIA results as less than 0.10 kU/L. Since the report from the reference lab using FEIA did 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.<sup>7</sup> In the Modified Class Scoring (MCS) system, 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 Ultra-Sensitive EIA System for Allergen-Specific IgE 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 FEIA. 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 the HYCOR Ultra-Sensitive EIA System'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 toward diagnosis and treatment planning for allergy patients.

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