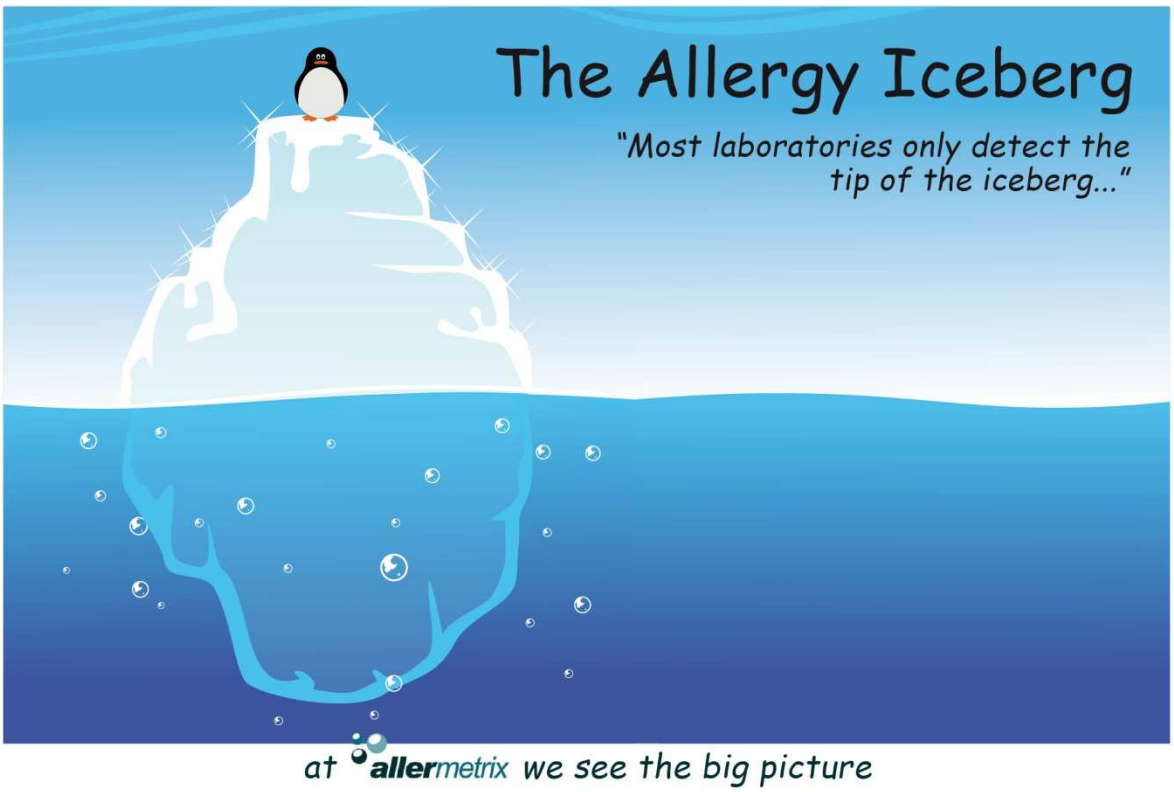

A Method Comparison Study

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Allermetrix has developed its own liquid allergen method that demonstrates greater sensitivity than solid-phase assays available today. It is calibrated to the WHO (75/502) primary reference standard and reports quantitative specific IgE results for over 400 different allergens.

As part of the validation of this assay, Allermetrix performed a dilution recovery analysis as well as a method comparison to Phadia ImmunoCAP. For the comparison study sera were split and tested at Allermetrix and a laboratory using the ImmunoCAP methodology within 2 – 3 days of one another. For dilution recovery analysis, all sample dilutions were made with equine serum. Results were used to evaluate the class to class agreement of the methods and quantitative nature of the Allermetrix assay.

Allermetrix sent out 137 tests on 25 different allergens to a laboratory that performs Phadia ImmunoCAP and compared results of Allermetrix Liquid Allergen to Phadia ImmunoCAP class scoring. In Tables 1a and 1b, the class comparison of the two techniques shows that over 94% of all results are within 2 classes and the Allermetrix class score is more often higher than that of Phadia. There are 8 results that are negative, Class 0, in ImmunoCAP, and are class 2 or 3 in the Allermetrix Liquid Allergen assay.

Some of the class differences between Allermetrix and Phadia are due to the differences in class cutoffs. Allermetrix classes are based on the modified RAST scoring system of Drs. Fadal and Nalebuff that match SET (skin endpoint titration) in allergic patients. The cutoff for positive in the Allermetrix liquid allergen system is 0.05 kU/L. Phadia ImmunoCAP classes are arbitrary cutoffs with positive set at 0.35kU/L or greater (based on correlation with nasal challenges in allergic patients). Solid phase assays suffer from low end insensitivity because of inherent non-specific binding, whereas liquid allergen non-specific binding is extremely low resulting in superior analytical sensitivity. Also, as one might expect, skin endpoint titration would yield higher sensitivity than nasal challenge as a threshold for positivity.

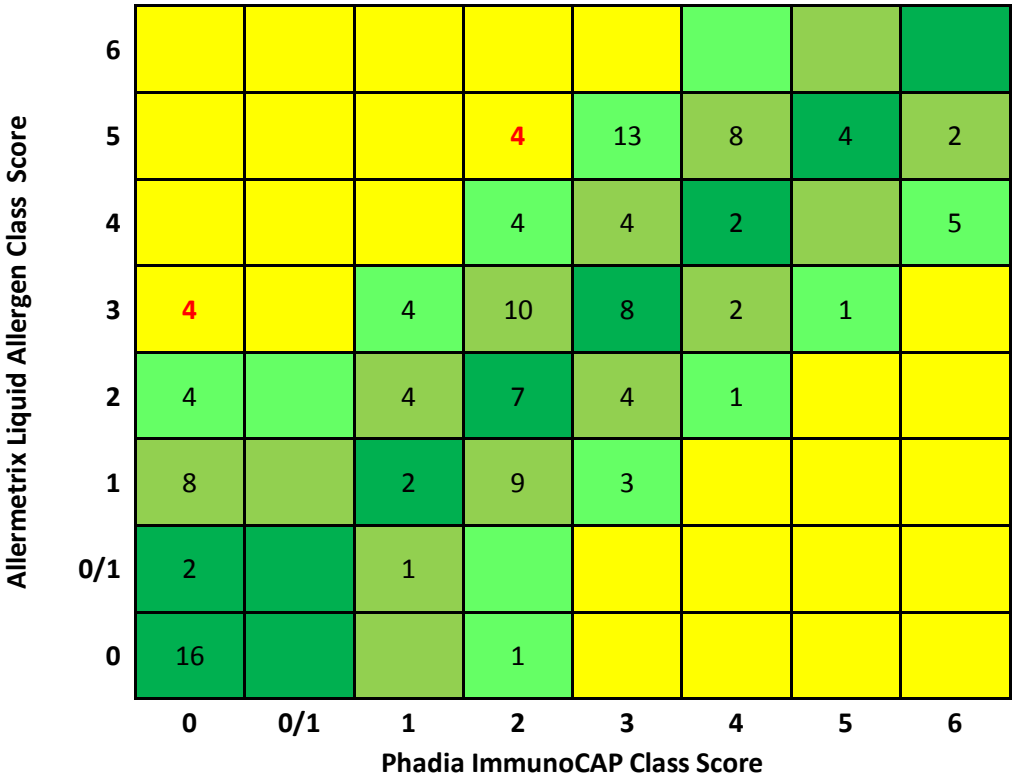

Table 1a Class Comparison of Allermetrix and Phadia

	n	%	cumulative percent
Total tests	137	100.00%	
same class match	41	29.93%	29.93%
+/- 1 Class	52	37.96%	67.88%
+/- 2 Classes	36	26.28%	94.16%
> 2 Classes	8	5.84%	

Table 1b Statistics of Class Comparison Data

Several positive samples were tested in the Allermetrix assay for dilution linearity. In Figure 1 it can be seen that samples on 17 different allergens were linear when diluted and demonstrate the quantitative characteristics of the assay.

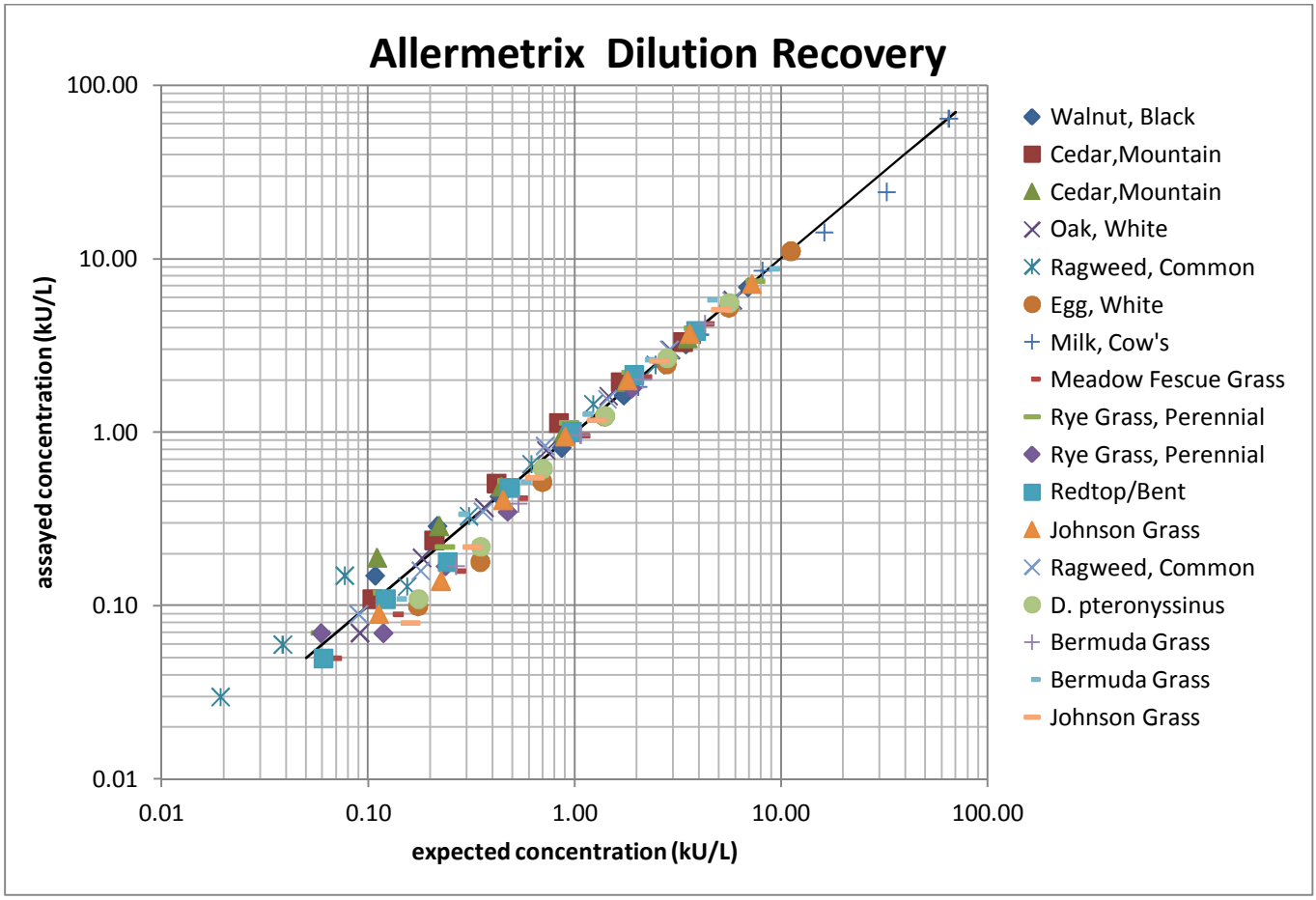

Figure 1 Dilution recovery of samples in the Allermetrix Liquid Allergen specific IgE assay.

Figure 2 shows that samples tested on 12 different allergens at Allermetrix diluted well below the 0.35 kU/L cutoff in the Phadia ImmunoCAP assay.

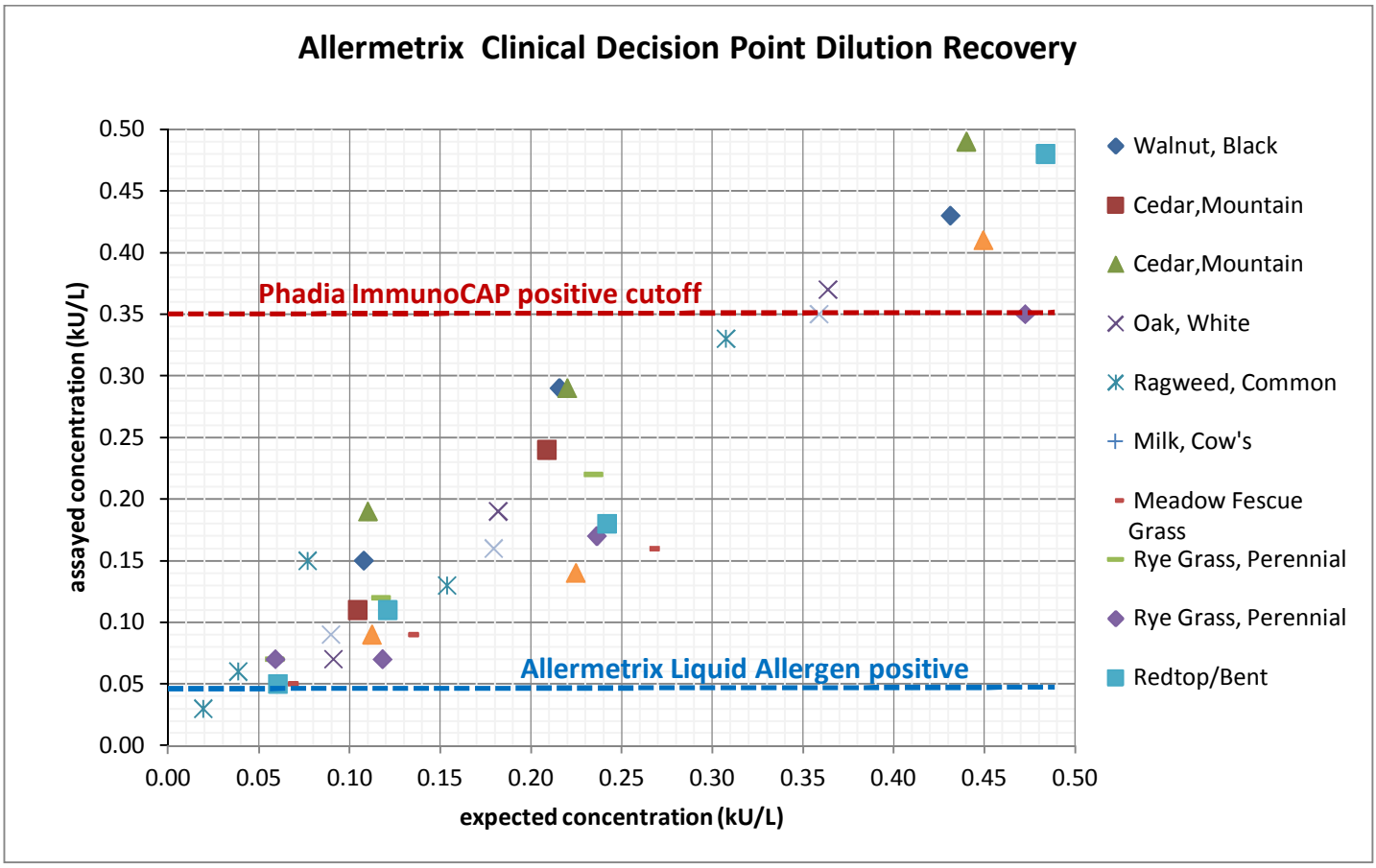

Figure 2 Low end dilution recovery for Allermetrix specific IgE assay.

Samples that were found to have similar specific IgE concentrations in both the Allermetrix Liquid Allergen and Phadia ImmunoCAP assay were diluted and tested in each assay. Samples assayed in the ImmunoCAP method were sent to the same laboratory that performed the class comparison. Figures 4a and 4b show that samples tested on ragweed, 5.47 and 6.54 kU/L, and mountain cedar, 3.52 and 3.92 kU/L, in Allermetrix and ImmunoCAP respectively had similar concentrations in both assays. Samples remained positive in the Allermetrix assay at a 32-fold dilution, while Phadia were positive only to the 8-fold dilution. Allermetrix Liquid Allergen assay reported positive results at 4-fold lower dilutions.

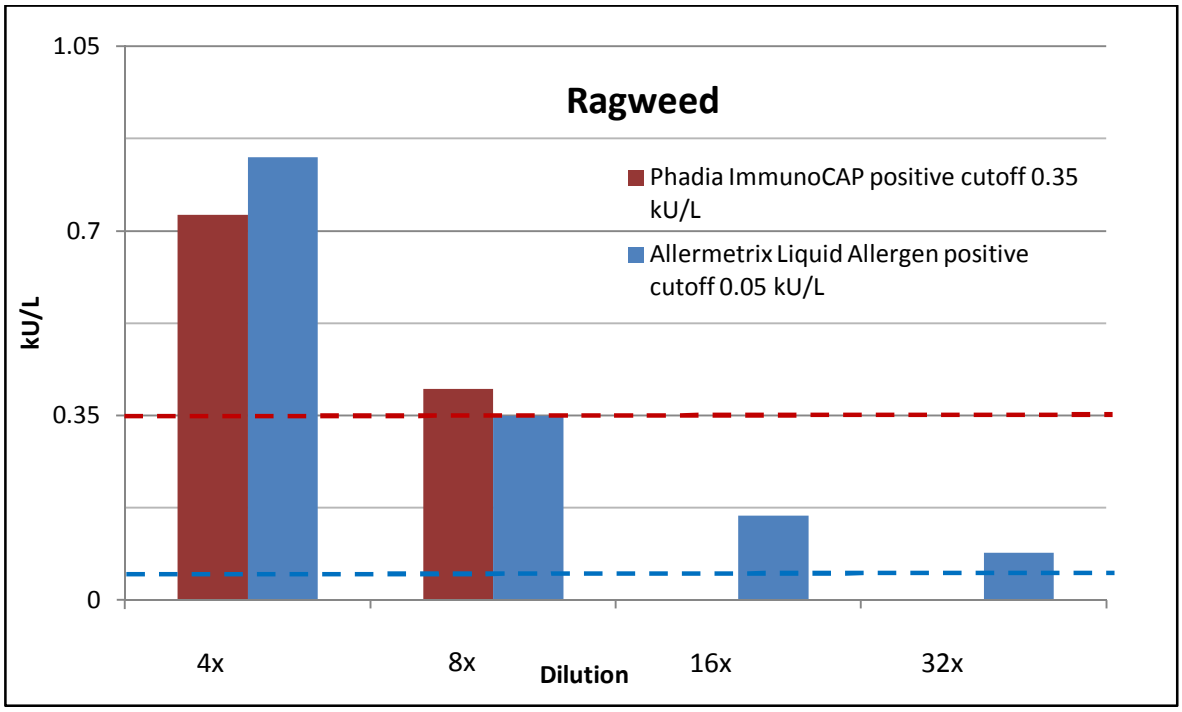

Figure 3a Ragweed low end dilution with Allermetrix Liquid Allergen and Phadia ImmunoCAP asasys.

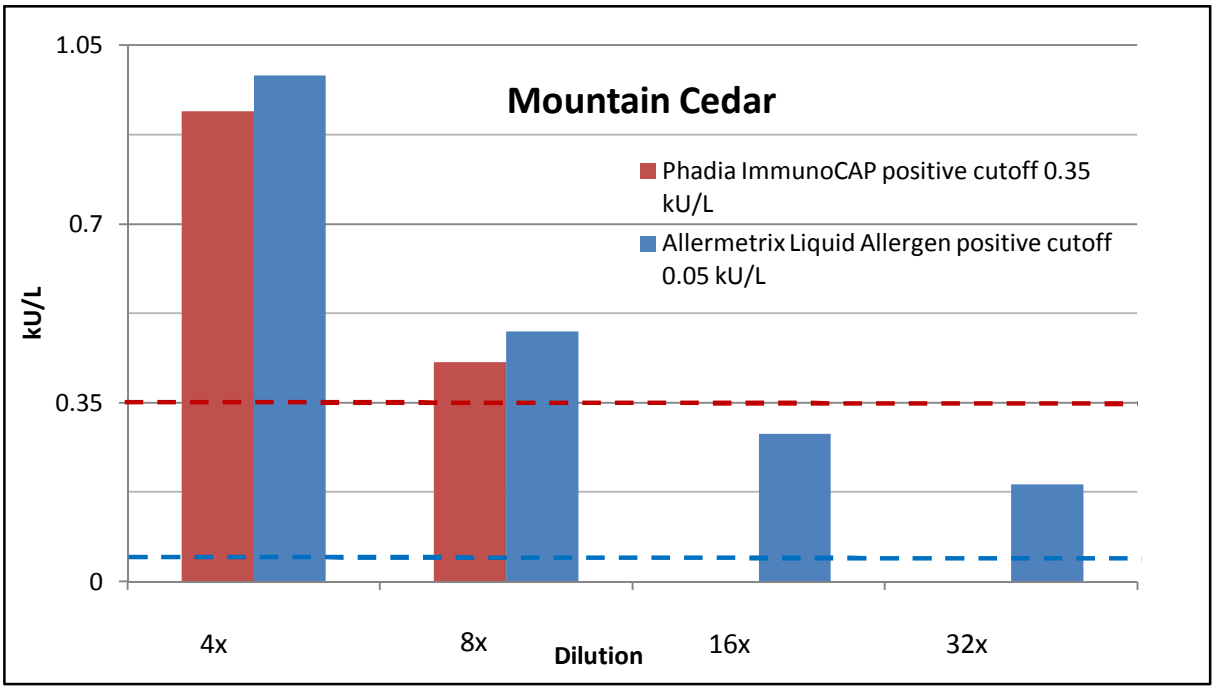

Figure 3b Mountain Cedar low end dilution with Allermetrix Liquid Allergen and Phadia ImmunoCAP assays.

The liquid allergen used in the Allermetrix assay presents the allergen in a natural conformation rather than denatured on a solid phase. Therefore in some cases the IgE antibodies may see allergen epitopes that are not well preserved on solid-phase tests.

Conclusion

The samples in the method comparison that were class 2 and 3 in Allermetrix Liquid Allergen and negative in Phadia ImmunoCAP may represent samples that are difficult to identify in solid-phase assays. The method comparison indicated that Allermetrix results are comparable to those of Phadia. Linear dilution recovery proves the quantitative nature of the Allermetrix method across the range of the assay, (0.05 – 100 kU/L). The parallel dilution recovery of samples with comparable concentrations of specific IgE demonstrate that Allermetrix specific IgE results are more sensitive where clinical decisions are made, at low concentrations of specific IgE. Testing with liquid allergens more closely matches skin testing due to greater sensitivity and can detect concentrations of specific IgE well below that of the standard solid phase assay.