

FULLY AUTOMATED IMMUNOASSAY SYSTEM FOR ALLERGY TESTING; EVALUATION OF IMMUNOCAP™ 1000 WITH FIVE SPECIFIC ALLERGENS

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Introduction

There are several immunoassay systems for in vitro allergy testing on the market. We have evaluated ImmunoCAP™ 1000, a fully automated random access immunoassay system from Pharmacia Diagnostics AB and compared the results to the ADVIA Centaur from Bayer Diagnostics. Both systems are calibrated to the WHO IgE IRP 75/702.

The design of the study made it possible to evaluate the following parameters: linearity (i.e. parallelism in dilution), precision and correlation of obtained concentrations between the two systems.

Four different pooled patient samples were tested undiluted and diluted 1/3, 1/9, 1/27 and 1/54. Each combination of sample and dilution was tested in 4 to 6 replicates for each of the following allergens: d1, e1, g3, w1 and w9.

The ImmunoCAP™ 1000 part of the study was performed at the Allergy Hospital in Helsinki, Finland and the ADVIA Centaur part was performed in a private laboratory in Germany.

Aim of the study

To evaluate the performance of the ImmunoCAP™ 1000 analyzer for *in vitro* measurement of specific IgE antibodies and compare the results to the ADVIA Centaur analyzer.

Results

The measured concentration ranged from 0.41 to 84 kU_A/L for the ImmunoCAP™ 1000 (in total 92 measurements, excluding 8 measurements that were below 0.35 kU_A/L), and from 0.36 to 47 kU_A/L for the ADVIA Centaur (excluding 22 measurements that were below 0.35 kU_A/L). Measurements below 0.35 kU_A/L were excluded from all calculations, but included in Figure 2 -7.

Precision

The concentrations were measured with a very high precision with the ImmunoCAP™ 1000; the pooled intra assay CV was below 3.5% over the whole measurement range (Figure 1, Table 2). The concentrations above 3.5 kU_A/L was measured with good precision with the ADVIA Centaur (6.0%), but concentrations below 3.5 kU_A/L showed poorer precision with the ADVIA Centaur (22.3%), Figure 1, Table 1.

Table 1. Pooled intra assay CV per system, in total and stratified for obtained concentrations below or above 3.5 kU_A/L (each measurement was based on 4-6 replicates, intra assay CV = CV within series of replicates).

System	Pooled intra assay CV (number of measurements)		
	Total	Below 3.5 kU _A /L	Above 3.5 kU _A /L
ImmunoCAP™ 1000	3.2% (92)	3.0% (44)	3.4% (48)
AD VIA Centaur	15.5% (78)	22.3% (35)	6.0% (43)

Linearity

The ImmunoCAP™ 1000 showed in general excellent dilution linearity for all tested allergens; d1, e1, g3, w1 and w9 (Figure 3 - 7).

The ADVIA Centaur showed good linearity in the high concentration level (> 3.5 kU_A/L). An unsatisfactory linearity appeared, however, in the lower concentrations with the allergens d1, g3 and w1 (Figure 3, 5 and 6).

Correlation

The relation of the obtained concentrations between the two systems was compared by linear correlation and by linear regression of the values of ImmunoCAP™ 1000 and ADVIA Centaur (Figure 2 and Table 2). The result shows a remarkably high number of samples which are detected positive in ImmunoCAP™ 1000 but detected as negative in ADVIA Centaur (17%). The correlation between the two systems was good (r>0.90) for e1, g3, and w9 (Table 3). For d1 and w1 the correlation is poorer depending on the non-linearity of the Advia Centaur in the lower regions (Table 2 and Figure 3 and 6).

Table 2. Linear correlation and linear regression of the values of the ADVIA Centaur on the values of the ImmunoCAP™ 1000 (based on the natural logarithm).

Allergen	Correlation	Slope	Intercept	N*
d1	0.89	0.93	-0.66	14
e1	0.97	0.91	0.43	17
g3	0.98	0.99	-0.48	19
w1	0.79	0.94	-0.75	11
w9	0.99	1.08	-0.01	15
Total	0.88	0.85	-0.04	76

*Number of paired measurements from the two systems with obtained concentrations of 0.35 kU_A/L or higher.

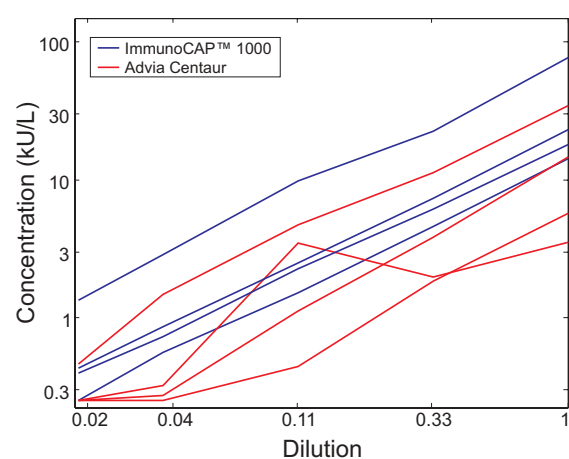


Figure 3. Linearity for allergen d1

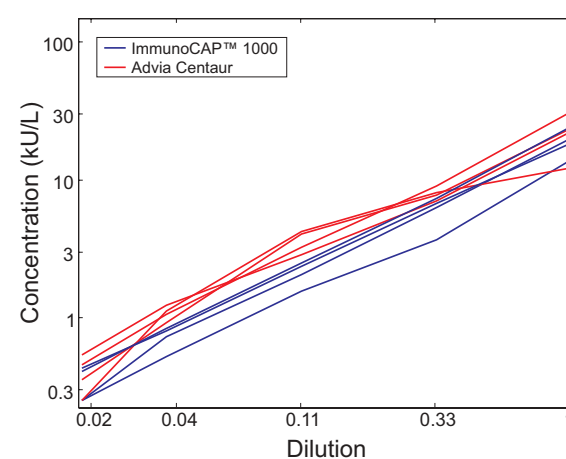


Figure 4. Linearity for allergen e1

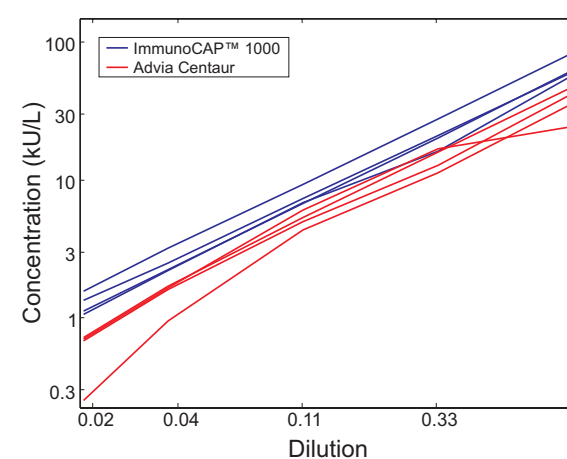


Figure 5. Linearity for allergen g3

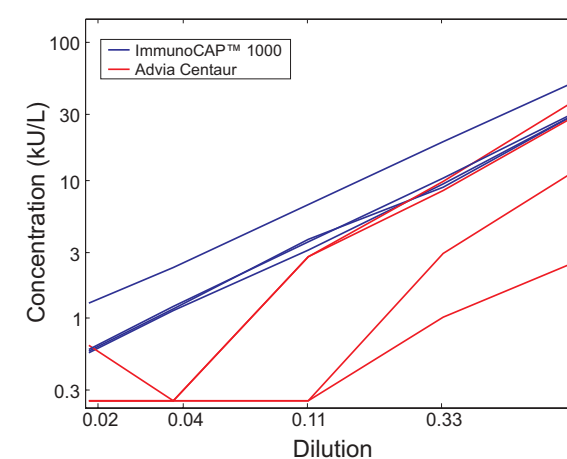


Figure 6. Linearity for allergen w1

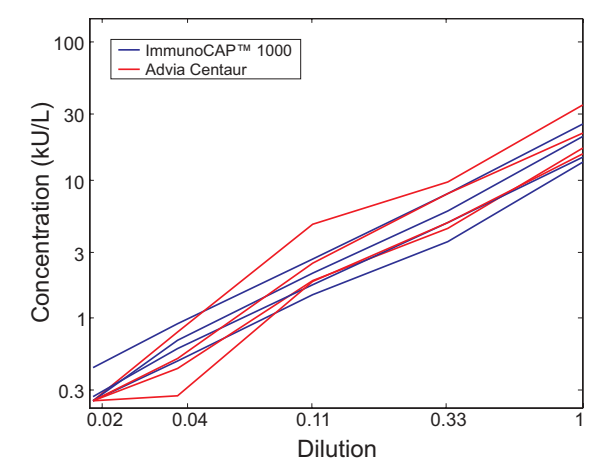


Figure 7. Linearity for allergen w9

Materials

Four different pooled patient samples were prepared and independently diluted with a pre-analyzed negative (<0.35 kU_A/L) serum pool in proportions 1/3, 1/9, 1/27 and 1/54; in total 100 combinations. The samples and their dilutions were divided into two identical sets, one for each analytical system. The set of the four samples with their dilutions were then tested according to the manufacturers instructions for all the five allergens (in total 100 measurements in 4-6 replicates, Table 3).

Table 3. - Allergens tested in the study.

d1	House dust mite – <i>Dermatophagoides pteronyssinus</i>
e1	Cat epithelium and dander
g3	Cocksfoot – <i>Dactylis glomerata</i>
w1	Common ragweed – <i>Ambrosia elatior</i>
w9	Plantain (English), Ribwort – <i>Plantago lanceolata</i>

Conclusion

The results from our study clearly shows that the ImmunoCAP™ 1000 from Pharmacia Diagnostics AB provides reliable and accurate results in the whole analytical range with the allergens tested. The easy handling and low hands-on time makes ImmunoCAP™ 1000 well suited for medium or large-sized laboratories.

The ADVIA Centaur performed less well regarding precision, especially below 3.5 kU_A/L. The ADVIA Centaur also showed an unsatisfactory linearity in several cases (d1, g3 and w1). In general, as an automated system ADVIA Centaur performed well.

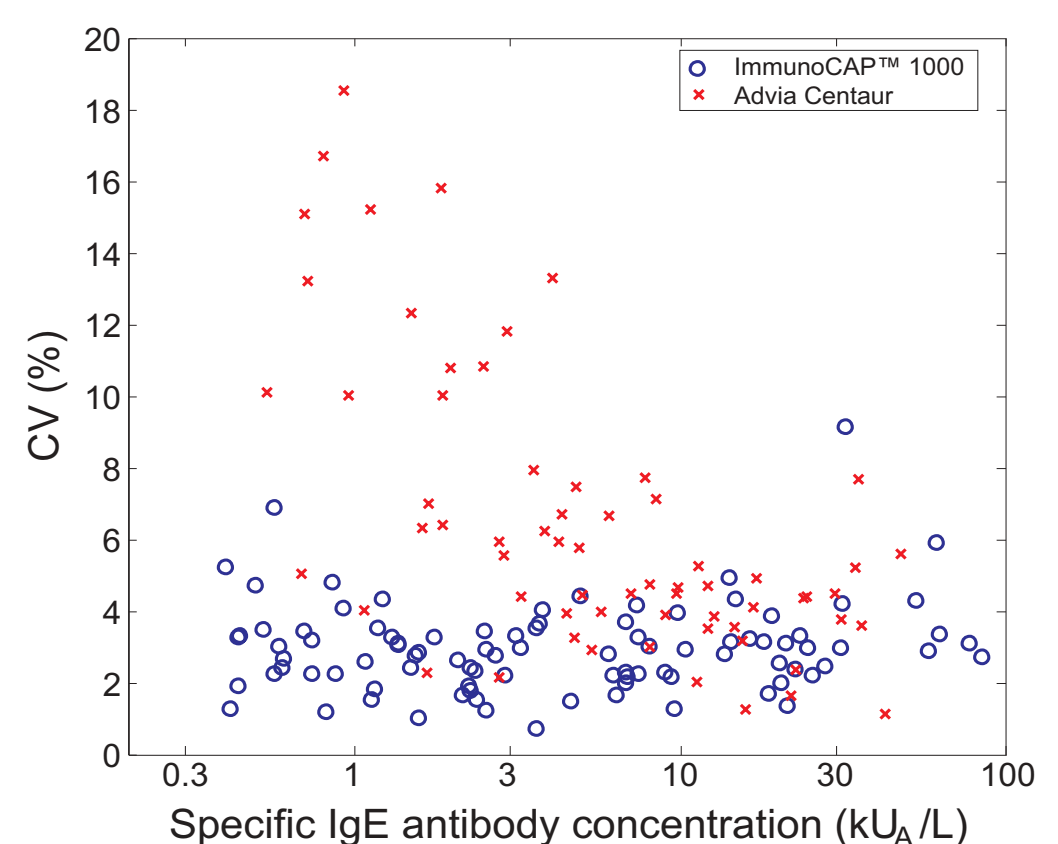


Figure 1. Precision profile for ImmunoCAP™ 1000 and ADVIA Centaur (13 ADVIA Centaur measurements with CV above 20% excluded from figure).

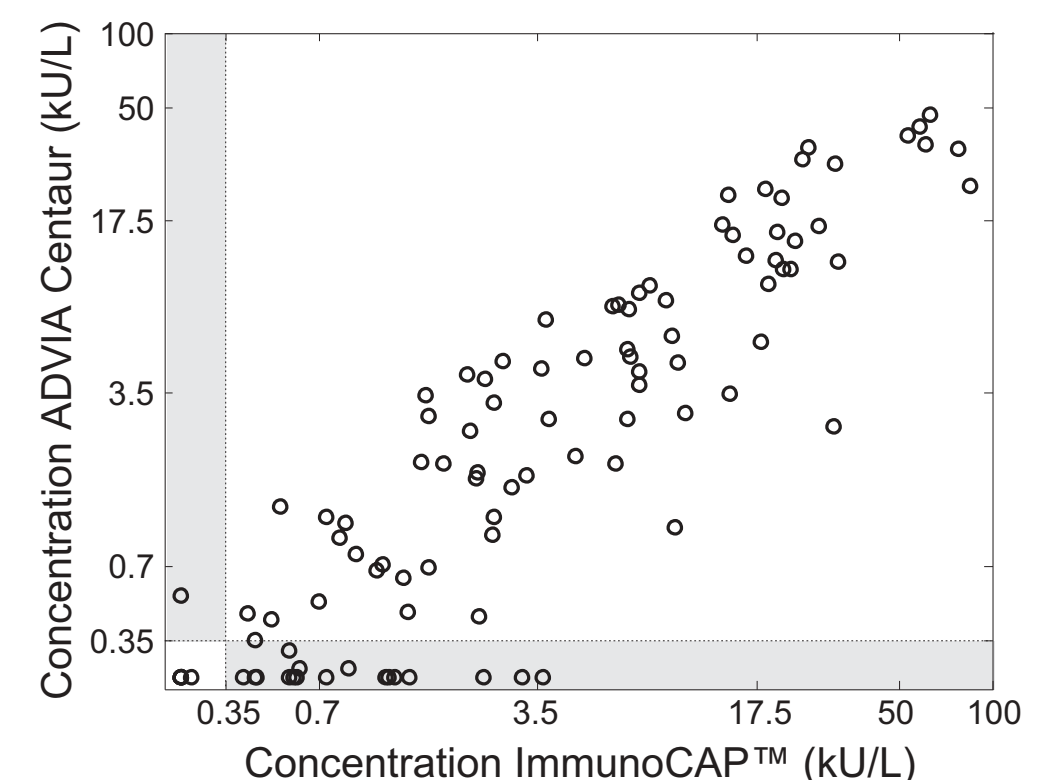


Figure 2. Correlation between ImmunoCAP™ 1000 and ADVIA Centaur

Methods

The Pharmacia ImmunoCAP™ 1000 is based on the well known ImmunoCAP immunoassay technology. Allergens are covalently coupled to a 3-dimensional porous cellulose solid phase with an extremely high binding capacity, encased in a capsule (ImmunoCAP). The detection system is based on an enzyme anti-IgE conjugate and a substrate, producing a fluorescence signal that is measured in the built-in fluorometer.

The ADVIA Centaur Specific IgE system is an IgE capture sandwich immunoassay, using streptavidin coated Paramagnetic Particles as solid phase, liquid biotin labeled allergens and chemoluminescence detection.

References:

Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities: Approved Guideline. I/LA 20-A, Vol. 17 No. 24.

Footnote:

The name of all UniCAP® products is being changed to the new brandname ImmunoCAP™.