

Letter to the Editor

In vitro determination of allergen-specific serum IgE. Comparative analysis of three methods

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Laboratory diagnosis of immunoglobulin E (IgE)-mediated allergic disease is based on quantification of allergen-specific serum IgE (sIgE). Second-generation assays (e.g., ImmunoCAP[®], Phadia, Uppsala, Sweden) are widely used, standardized and reliable. By and large, they correspond to skin testing (1). More recently, third-generation assays that allow workstation consolidation have been introduced (e.g., Immulite 2000[®], Diagnostic Products Corporation, Los Angeles, CA, US, and Advia Centaur[®], Bayer, Dublin, Ireland). Two studies evaluated the Immulite 2000 and concluded that this system was equivalent to or better than the ImmunoCAP (2, 3). These studies focused on *Dermatophagoides pteronyssinus*, *D. farinae*, cat, grass, birch, mugwort, honey bee venom (HBV), yellow jacket venom (YJV) (2), and on inhalant and food screens, *D. pteronyssinus*, cat, horse, and dog (3). The technical characteristics of the Immulite 2000 have already been described (4). The Advia Centaur has been reported to compare favorably with the ImmunoCAP for milk, egg, timothy grass, Bermuda grass, dust mites, and cat (5).

In an international external quality assessment (EQA) scheme with participants from Belgium, The Netherlands, Portugal and Finland, we observed substantial differences between the three methods for peanut (f13), HBV (i1), YJV (i3) and latex (k82). For example, mean values for peanut were 2.81, 0.64 and 0.37 kU/L for the ImmunoCAP (n=194), Immulite (n=87) and Advia (n=15) systems, respectively.

To prepare EQA samples, single donations were used from allergic volunteers selected by an allergo-

logist. Samples were collected according to the NCCLS37A procedure, dispensed into 0.5-mL aliquots and frozen. No additives were used, which implies that the samples were 100% human and native. Spare samples were frozen at –84°C. Comparison of the results for allergic testing in fresh samples and frozen samples stored for 3 years showed no differences, indicating the stability of the material.

The findings prompted us to perform a retrospective study in which we compared the outcome of the three assays with anamnesis and a skin test.

Circulating sIgE was measured using ImmunoCAP 1000, Immulite 2000, and Advia Centaur systems according to manufacturer's instructions. Results >0.35 kU/L for the ImmunoCAP and Advia and >0.1 kU/L for the Immulite were scored as positive. Skin testing was performed according to standard procedures (6) using allergen extracts from ALK-Abello (Hørsholm, Denmark) (HBV, YJV, peanut), and Stalergènes (Antony, France) (latex). Anamnesis was documented by experienced allergists. The samples were selected based on historical positive test results (ImmunoCAP) for either peanut, HBV, YJV or latex. Selection of samples was guided by clinical history.

We determined sIgE to peanut in 11 patients, to latex in nine patients, and to HBV and YJV (n=21) in 22 patients with insect sting anaphylaxis. We thus performed 63 sIgE determinations in 42 different samples.

The ImmunoCAP, Immulite and Advia methods revealed sIgE for peanut in 11, 10 and 0 of the 11 samples, respectively. Only three of the 11 patients had a positive skin test and anamnesis. The methods revealed sIgE for latex in eight (ImmunoCAP), eight (Immulite) and seven (Advia) of nine samples. In six of these nine patients, a positive skin test and anamnesis were documented. The methods revealed sIgE for HBV in 16 (ImmunoCAP), 14 (Immulite), and eight (Advia) of 22 patients. Only nine of the 22 patients had a positive HBV skin test and positive anamnesis for HBV allergy. The methods revealed sIgE for YJV in 18 (ImmunoCAP), 17 (Immulite) and 15 (Advia) of 21 samples. In 16 of these 21 patients, a positive YJV skin test and positive anamnesis for YJV allergy were documented.

Combining the four allergens, the ImmunoCAP and Immulite systems detected sIgE antibodies in all 34 determinations for which both anamnesis and a skin test revealed allergic disease. The Advia method, in contrast, was negative for seven of these 34 determinations (3 peanut, 1 latex, 1 HBV, 1 YJV). This was significantly different from the ImmunoCAP and

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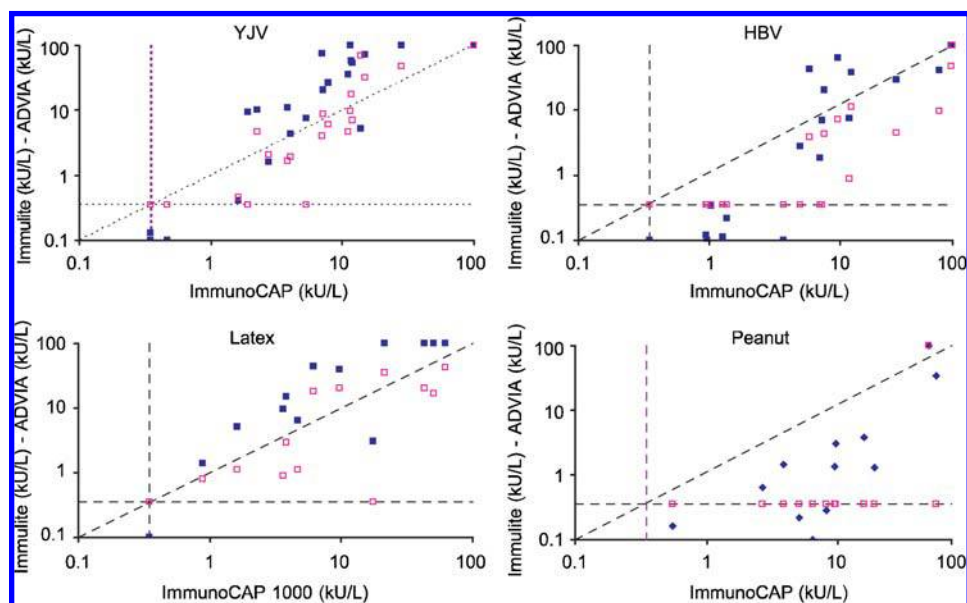


Figure 1 Scatterplots of Immulite (filled symbols) and Advia Centaur (open symbols) (y-axis) vs. ImmunoCAP 1000 (x-axis) results for HBV, YJV, peanut and latex.

Table 1 Prevalence and specification of discrepancies between skin test and three in vitro tests.

Allergen	Prevalence of discrepancies		
	All negative	All positive	Discrepancy
Peanut	0	0	11 (100%)
Latex	1	5	3 (33%)
Honey bee venom	5	8	9 (41%)
Yellow jacket venom	2	14	5 (24%)

Allergen	Specification of discrepancies				
	Clinical tests		Laboratory tests		
	Anamnesis	Skin test	ImmunoCAP	Immulite	ADVIA
Peanut	–	–	+	+	–
	–	–	+	+	–
	–	–	+	+	–
	–	+	+	+	–
	–	+	+	+	–
	–	+	+	+	–
	–	+	+	–	–
	–	?	+	+	–
	+	+	+	+	–
	+	+	+	+	–
Latex	–	–	+	+	+
	–	–	+	+	+
	+	+	+	+	–
Honey bee venom	+	+	+	+	–
	–	+	+	+	–
	?	+	+	+	–
	?	+	+	+	–
	?	+	–	–	–
	?	+	+	+	–
	?	–	+	+	–
	?	–	+	–	–
Yellow jacket venom	+	+	+	+	–
	+	+	+	+	–
	?	–	+	+	+
	?	–	+	–	–
	?	+	–	+	–

? indicates inconclusive anamnesis or skin test.

Immulite methods ($p=0.016$; χ^2), suggesting that the Advia is less sensitive than the ImmunoCAP and Immulite methods.

The ImmunoCAP and Immulite methods revealed sIgE in five of eight determinations for which both anamnesis and skin test were negative for allergic disease (3 peanut, 2 latex). The Advia gave a positive test result for two of the eight determinations (latex). The ImmunoCAP, Immulite and Advia systems revealed sIgE in five, three, and zero of 10 samples (7 HBV, 3 YJV) for which the skin test was negative and anamnesis inconclusive (HBV or YJV contact not documented). Together, the ImmunoCAP and Immulite methods detected specific IgE antibodies in 10 and eight of 18 samples for which the skin test was negative and anamnesis negative/inconclusive. The Advia result, in contrast, was positive in only two of these 18 determinations. This was significantly different from the ImmunoCAP ($p=0.0373$, χ^2) and suggests that the Advia is more specific than the ImmunoCAP method.

The presence of sIgE antibodies in the absence of a positive skin test might be caused by cross-reactivity with carbohydrate determinants (7, 8). This was substantiated by a positive sIgE test for bromelain in five of five samples obtained from patients with negative skin test, negative anamnesis and positive IgE antibodies. Mari (9) reported that, particularly for peanut, high levels of sIgE antibodies correlated with cross-reactive carbohydrate determinants.

Scatterplot results for HBV, YJV, peanut and latex are shown in Figure 1.

Major differences between the three instruments were observed for peanut. ImmunoCAP values were higher than Immulite values, whereas Advia gave almost no positive results. For YJV and latex, Immulite gave higher results than ImmunoCAP and Advia. For HBV, Advia gave lower results than ImmunoCAP and Immulite. Similar findings for the Immulite have been reported for YJV and HBV (3).

We compared three in vitro systems with skin test and anamnesis for detection of sIgE to peanut, latex, HBV, and YJV. Many discrepancies were observed, especially for peanut (Table 1). The ImmunoCAP and Immulite systems are more sensitive but less specific than the Advia.

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