



# Evaluation of IMMULITE® 2000 Allergy in a Reference Laboratory

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In 2002, Laboratoire Marcel Mérieux (LMM), a private laboratory in France, sought to increase its workflow, consolidate instruments and decrease labor costs in allergy testing. It conducted an analytical performance study of IMMULITE® 2000 Allergy. Upon testing 2,115 patient samples using 28 inhalant allergens and the AlaTOP® inhalant screening panel, IMMULITE 2000 Allergy demonstrated good reproducibility, binding capacity and specificity. Study results demonstrated a 96.6% agreement between IMMULITE 2000 AlaTOP and Phadiatop®. Results also indicated a total agreement of 89% between IMMULITE 2000 Allergy and the Pharmacia CAP system, with 86% agreement within one class. Pleased with its findings, LMM incorporated the IMMULITE 2000 as part of its instrumentation.

LMM's Allergy Unit performs 700 to 900 specific-IgE assays daily. At the time of the study, LMM had eight technicians on staff, and its allergy testing instruments included eight AutoCAP systems, two UniCAP® systems, a Tecan for sample distribution, a positioning guide for the ImmunoCAP™ distribution, and a gamma counter. In considering IMMULITE 2000 Allergy for its facilities, LMM was seeking the following benefits:

- Consolidation onto fewer instruments
- Full automation (automating allergen pipetting)
- Decreased labor costs
- Improved workflow
- Enhanced safety and reliability

The laboratory's internal protocol defined the IMMULITE 2000 Allergy study as being strictly analytical in an effort to produce an unbiased comparison, since no clinical data were available and there is no gold standard for in vitro allergy diagnosis.

Precision, linearity under dilution and specificity were evaluated according to US NCCLS guidelines. The protocol also included a method comparison of IMMULITE 2000 Allergy to the Pharmacia CAP system. The most routinely run allergens were tested, including 28 inhalant allergens (mites, house dust, latex, tree/weed/grass pollens, venoms, cockroach, molds and animal dander/epithelium) that accounted for 70 percent of the total inhalant allergen requests at LMM, and the AlaTOP inhalant screening panel. See Table 1.

Samples were randomly selected during the first three months of the study from samples routinely received by the laboratory. Some positive samples generated by the Pharmacia CAP system were added to increase the ratio of positive to negative results.

<b>Mites and House Dust</b> • D1: Der. pteronyssinus • D2: Der. farinae • H2: Hollister-Stier <b>Occupational</b> • K82: Latex <b>Tree Pollens</b> • T3: Birch • T7: Oak • T15: White Ash • T9: Olive • T23: Italian Cypress <b>Weed Pollens</b> • W1: Common Ragweed • W6: Mugwort • W9: English Plantain • W21: Parietaria judaica	<b>Grass Pollens</b> • G3: Orchard Grass • G5: Perennial Rye Grass • G12: Cultivated Rye Grass <b>Venoms</b> • I1: Honey Bee Venom • I3: Yellow Jacket Venom • I4: Paper Wasp Venom • I6: Cockroach <b>Molds</b> • M3: Aspergillus fumigatus • M6: Alternaria tenuis <b>Animals</b> • E1: Cat Dander Epithelium • E2: Dog Epithelium • E3: Horse Dander • E5: Dog Dander • E6: Guinea Pig Epithelium • E82: Rabbit Epithelium
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Table 1.

Results were as follows:

### Precision

- Intraassay: CVs ≤ 5%.

Intraassay			
Allergen	Number of tests	Mean kU/L	CV (%)
D1	11	0.88	4.1
M6	12	1.9	5
G3	12	12.1	4.1

Table 2.

- Interassay: CVs ≤ 17% at low specific-IgE concentrations and < 10% at moderate and high specific-IgE concentrations.

Interassay			
Allergen	Number of tests	Mean kU/L	CV (%)
G3	17	0.69	17.1
D1	18	18.0	9.7
E1	21	42.7	8.3

Table 3.

- A regression analysis of lot-to-lot reproducibility, with 102 samples and 7 antigens, demonstrated a slope of 1.0271 and an R<sup>2</sup> of 0.9566.

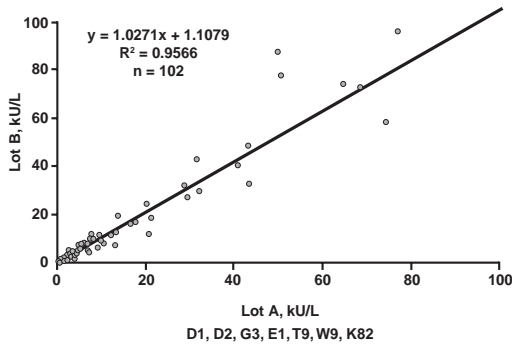


Figure 1.

### Linearity Under Dilution

- An R<sup>2</sup> of > 0.97 was demonstrated for 30 positive serum samples against 6 allergens (D1, D2, E1, G3, K82 and T3) when diluted down to 0.316 kU/L at dilution factors ranging from 1 to 64.

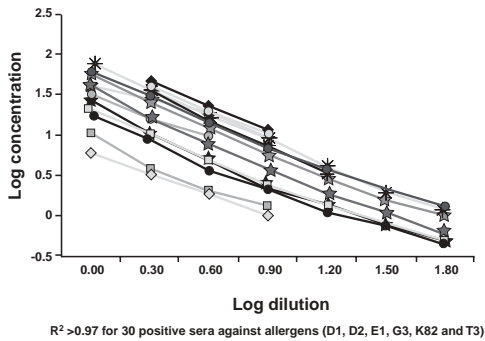


Figure 2.

- A regression analysis comparing observed to expected values for 25 samples against 6 allergens showed good linearity, with a slope of 0.9052 and an R<sup>2</sup> of 0.9344.

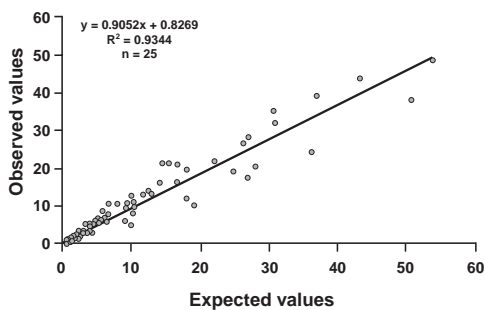


Figure 3.

### Method Comparison

- AlaTOP vs. Phadiatop demonstrated 96.6% agreement.

N = 320

		AlaTOP		
		-	Border	+
Phadiatop	-	202	17	7
	Border	8	5	3
	+	4	2	72

Agreement = 96.6%

Border = borderline result

AlaTOP: cutoff value ± 10%

Phadiatop: from 0.35 to 0.59 kU/L

Table 4.

- IMMULITE 2000 Allergy vs. Pharmacia CAP: results from all allergens tested on 2,115 samples demonstrated a total agreement of 89%, with 86% agreement within one class (Figure 8). Many individual allergens showed even greater total agreement. The mold allergen *Alternaria tenuis*, for example, had a total agreement of 96%, with 96% agreement within one class.

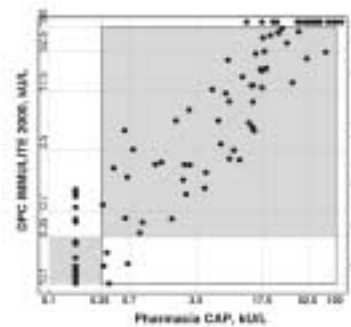


Figure 4.

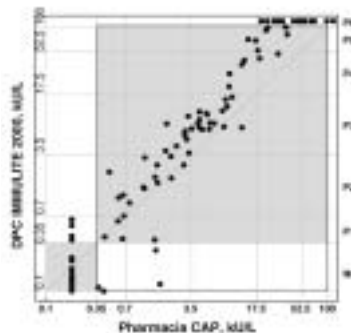
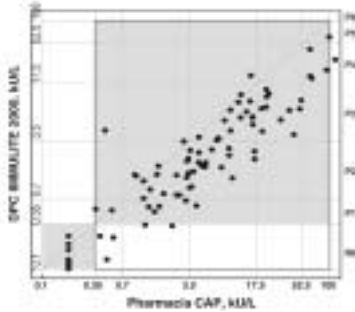
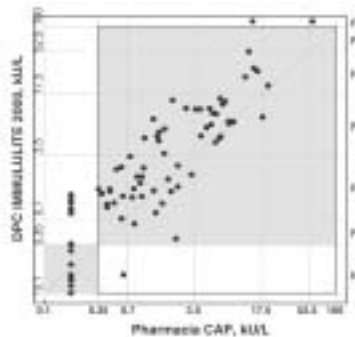


Figure 5.



M6: *Alternaria tenuis*  
 n = 127  
 Total agreement: 96%  
 One class identical: 96%

Figure 6.



I3 : Yellow Jacket Venom  
 n = 85  
 Total agreement: 89%  
 One class identical: 91%

Figure 7.

### Specificity

Inhibition experiments performed with Stallergènes extracts demonstrated good results. Where IMMULITE 2000 Allergy results were positive and CAP system results were negative, 15 out of 29 samples demonstrated >72% inhibition. Inhibition was >91% in several cases. Where IMMULITE 2000 Allergy results were negative and CAP system results were positive, 14 out of 76 cases demonstrated >65% inhibition (except for the insect allergens I1, I4 and I6, which were below 42%).

Results of the evaluation were presented in poster format at CORATA (October 2002), JPA (January 2003) and EAACI (June 2003). Their findings led LMM to conclude that IMMULITE 2000 Allergy offers good reproducibility, binding capacity and specificity. In addition, results correlate well with Pharmacia's Phadiatop and CAP system. They also determined that IMMULITE 2000 Allergy would allow for routine use and easy integration in the laboratory.

### Total Agreement

			3	2	1	44	
		2		15	6	60	
	1	10	26	24	28	83	
CAP RIA	9	10	120	158	89	34	19
	67	67	219	90	7		1
	85	38	68	8			
	650	39	28	1			

IMMULITE 2000

N = 2115  
 Total agreement = 89%  
 One class identical = 86%

Figure 8.