

Allergy testing on the IMMULITE 2000 Random-Access immunoanalyzer – a clinical evaluation study

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Abstract

Purpose: We aimed to evaluate the diagnostic performance of the IMMULITE 2000 Allergy System from Diagnostic Products Corporation (DPC) for the detection of inhalant and food allergies, focusing on inhalant and food screens, mixes and single allergens.

Methods: Serum samples were collected from new, unselected patients who were referred to the allergist for a suspected allergy. Patients were classified as study diagnosis-positive for inhalant (food) allergy if they had both a positive clinical examination/history and a positive skin test for inhalant (food) allergy; otherwise – failing one or both of these criteria – they were classified as study diagnosis-negative. Classification and testing of the serum samples was carried out in a blinded fashion. Values greater than 0.35 kU/L were considered positive.

Results: Of the 118 patients included, 63 were considered study diagnosis-positive for inhalation and/or food allergy. DPC inhalation screening showed 82% total agreement (TA) and 91% sensitivity relative to the study diagnoses. The DPC food panel showed 96% TA and 98% specificity relative to the study diagnoses. Relative to specific intracutaneous testing (ICT), the DPC D1, E1 and E5 assays had sensitivity of 82–90%; tree and grass panels had sensitivity of 74% and 95%. The DPC weed panel and initial lots of DPC E5 had poor sensitivity (< 40%); mold panel sensitivity was equally low for both DPC and the routinely used Pharmacia assay (36%). Relative to skin prick testing (SPT), specific food allergens had TA of 94–98% and specificity of 95–100%.

Conclusion: In patients classified by the combination of clinical examination/history and skin test results, the DPC IMMULITE 2000 Allergy System generally demonstrated acceptable sensitivity, specificity and TA compared to the study diagnoses, both at the screening level and at the level of panels and single allergens.

Keywords: allergen-specific IgE; diagnostic performance; food allergy; inhalation allergy; skin tests.

Introduction

By the end of the 20th century, it was clear to experts in the field of allergy diagnosis and management that the quality of in vitro tests for allergen-specific immunoglobulin E (sIgE) had undergone a clinically significant evolution since the advent of Phadebas RAST® in 1974 as a consequence of major technical improvements (1–4). Indeed, so striking were these improvements that the experts adopted terms such as “early” and “modern” or – more commonly and more aptly – “first generation” and “second generation” to distinguish between the original radioallergosorbent (RAST) technology and the state-of-the-art systems being offered some 25 years later by the major manufacturers of sIgE blood tests, including Pharmacia, Diagnostic Products Corporation (DPC) and others (1–6). The multifaceted technical advances culminating in second-generation assay systems for sIgE offered increased turnaround times, enabled automation and batch processing on dedicated instruments, employed enzyme substrates and photometry or fluorimetry for signal detection, used multipoint calibration curves for interpolation and generated quantitative units traceable to the WHO International Reference Preparation 75/502 for Human IgE. They continued to employ the same cutoff, 0.35 kU/L, for distinguishing positive from negative results. Their clinical significance lay above all in the fact that the experts were finally prepared to embrace testing for sIgE in blood by a validated, second-generation assay system, as essentially on a par with skin testing in the established paradigm for diagnosing IgE-mediated allergic disease (1–4).

Recently, third-generation sIgE assays that present interesting analytical characteristics and allow workstation consolidation of in vitro diagnostic allergy testing with endocrinology and tumor marker testing have been introduced. Among them are the ADVIA Centaur from Bayer and the IMMULITE 2000 from DPC (7). In our hospital setting, we aimed to investigate specifically whether the third-generation IMMULITE 2000 Allergy System with design features such as liquid-phase allergens, monoclonal detection, use of an actual zero calibrator allowing an extended measurement range – with a detection limit of 0.10 kU/L and a functional sensitivity of 0.20 kU/L – and a time-to-first-result of approximately 1 hour, could be regarded as an acceptable candidate for replacing the UniCAP 100, a second-generation system for sIgE from Pharmacia (8–10) routinely used by us at the time of the study. To this end, we aimed to evaluate whether the diagnostic accuracy of the IMMULITE 2000 Allergy System in clinical practice was, on balance, at least as good as that of the system

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routinely used in our institution at the time of the study.

Materials and methods

Systems and assays

The DPC IMMULITE® 2000 Allergen-Specific IgE and IMMULITE 2000 AlaTOP® Allergy Screen are enzyme-enhanced chemiluminescent assays developed for use on the IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA), a fully automated, random-access analyzer that allows 138 liquid allergens to be stored on board. During the assay, ligand-labeled allergen and a 50-μL sample of patient serum are incubated with an anti-ligand-coated bead for 30 min. After an intermediate spin wash cycle, alkaline phosphatase-labeled anti-IgE monoclonal antibodies are incubated with the allergen-specific IgE captured on the bead for another 30 min. A chemiluminescent substrate is added after the final spin wash and incubated for 5 min with shaking before measuring the chemiluminescence and reporting results. The time-to-first-result is thus 65 min. The analytical performance of the IMMULITE 2000 Allergy has been the subject of at least two published studies (11, 12).

The Pharmacia UniCAP® 100 Allergen-Specific IgE and UniCAP Phadiatop are fluoroenzymometric assays for use on the semiautomated UniCAP 100 batch analyzer (Pharmacia, Uppsala, Sweden) (8–10). A 40-μL sample of patient serum is incubated with an allergen, which is covalently coupled to the ImmunoCAP. After washing the ImmunoCAP, enzyme-labeled anti-IgE is added to form a complex. Following this incubation, unbound enzyme-labeled anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. Total time for a single batch (assay run) is 150 min.

In addition to the specific anti-IgE reagents, assays for the measurement of specific IgE antibodies require the determination of a standard curve for reporting results quantitatively in kU/L. Calibrators in both the IMMULITE 2000 and UniCAP 100 assay systems measure in kU/L and are traceable to the WHO International Reference Preparation 75/502 for Human IgE.

In vitro diagnostic screenings, panels and single allergens tested

For the purpose of the evaluation, we focused on a spectrum of inhalant and food allergens representing a significant fraction of the Allergy Department's typical workload in a large peripheral hospital setting like ours. According to the historical test request database of the clinical chemistry laboratory, the most frequent single allergen and allergen panel requests in the Breda-Oosterhout region are D1, E1, E3, E5, Gp3, Mp1, Wp3 and Tp9 for inhalant allergy, and F1, F2, F3, F4, F13 and F14 for food allergy. Accordingly, these were all evaluated as a fixed battery of screenings, panels and single allergens (see below) in coded serum samples from all patients included, irrespective of the specific test requests made by the allergist. In addition, a test for total circulating IgE was also performed for each patient.

AlaTOP (for the IMMULITE 2000 system) and Phadiatop (for the UniCAP 100 system) are multiallergen screening tests each containing several inhalant allergens. The composition of the DPC AlaTOP is as follows: D1, house dust mite (*Dermatophagoides pteronyssinus*); E1, cat dander epithelium; E5, dog dander; G2, Bermuda grass (*Cynodon dactylon*); G6, Timothy grass (*Phleum pratense*); M1, *Penicillium notatum*; M6, *Alternaria alternata* (*Alternaria tenuis*); T3, birch (*Betula verrucosa*); T17, Japanese cedar (*Cryptomeria japonica*); W1, common ragweed (*Ambrosia elatior*); W9, English plantain (*Plantago lanceolata*); and W19, wall pellitory (*Parietaria officinalis*). Note that AlaTOP does not include horse dander, E3. According to Pharmacia, Phadiatop is not a mixture test but an atopy test, which recognizes most atopic subjects by using major allergen components. Pharmacia does not provide further information on the exact composition of Phadiatop.

Allergen panels (mixes) used in the fixed battery of serological tests on IMMULITE 2000 and UniCAP 100 were as follows:

- Grass panel 3 (Gp3, Gx3) contains: G1, sweet vernal grass (*Anthoxanthum odoratum*); G5, perennial rye grass (*Lolium perenne*); G6, Timothy grass (*Phleum pratense*); G12, cultivated rye grass (*Secale cereale*); and G13, velvet grass (*Holcus lanatus*).
- Mold panel 1 (Mp1, Mx1) contains: M1, *Penicillium notatum*; M2, *Cladosporium herbarum*; M3, *Aspergillus fumigatus*; M5, *Candida albicans*; and M6, *A. tenuis*.
- Tree panel 9 (Tp9, Tx9) consists of: T2, alder (*Alnus incana*); T3, birch (*Betula verrucosa*); T4, hazel (*Corylus avellana*); T7, oak (*Quercus alba*); and T12, willow (*Salix caprea*).
- Weed panel 3 (Wp3, Wx3) contains: W6, mugwort (*Artemisia vulgaris*); W9, English plantain (*Plantago lanceolata*); W10, lamb's quarter (*Chenopodium album*); W12, goldenrod (*Solidago virgaurea*); and W20, nettle (*Urtica dioica*).
- Food panel 5 (Fp5, Fx5) consists of: F1, egg white; F2, cow's milk; F3, codfish (*Gadus morhua*); F4, wheat (*Triticum aestivum*); F13, peanut (*Arachis hypogaea*); and F14, soybean (*Glycine max*).

These IMMULITE 2000 panels and UniCAP mixes have essentially the same composition, except that the Pharmacia Mold Mix 1 does not include *Candida albicans* (M5).

Specific allergens used in the fixed battery of serological tests in this study by both the IMMULITE 2000 and UniCAP 100 systems were:

- For inhalants: D1, house dust mite (*Dermatophagoides pteronyssinus*); E1, cat dander epithelium; E3, horse dander; and E5, dog dander.
- For foods: F1, egg white; F2, cow's milk; F3, codfish (*Gadus morhua*); F4, wheat (*Triticum aestivum*); F13, peanut (*Arachis hypogaea*); and F14, soybean (*Glycine max*).

For the quantitative allergen-specific IgE tests and the panels (or mixes), a positive result was defined as a concentration ≥ 0.35 kU/L, equivalent, by the standard scoring system, to a result of class 1 or higher. The inhalant screening tests are qualitative assays. The DPC AlaTOP is positive when the IMMULITE 2000 instrument response (in counts per second) is greater than 2.5-fold the response of the assay's low adjustor. The reference for the Pharmacia Phadiatop is the specific IgE calibrator of 0.35 kU/L; a patient sample with a fluorescence response higher than or equal to that of the reference indicates a positive result.

Allergen extracts and skin testing procedures

Allergen extracts for skin testing [intracutaneous test (ICT) and skin prick test (SPT)] were purchased from ALK-Abello BV (Nieuwegein, The Netherlands); standard packages were used. The single allergens and mixes generally used by the

allergist in cases of suspected inhalation and food allergy are described in Routine patient work-up by the allergist.

In The Netherlands, performing ICT for inhalant allergy testing is still standard practice (13). The reasons for this are that ICT is more reproducible and approximately 1000–10,000-fold more sensitive than SPT (14, 15), notwithstanding the fact that ICT causes more systemic side effects and more false-positive reactions than SPT.

We do not use ICT testing for food allergy because the regular manufacturer, ALK-Abello, delivers only SPTs for food allergy. ICT and SPT were performed according to Dreborg's guidelines for skin testing (16). A positive ICT was defined as a wheal diameter $\geq 70\%$ of the histamine control diameter; a positive SPT was defined as a wheal diameter ≥ 3 mm (13–15).

Study population

A prospective clinical study was set up to include unselected Caucasian adolescents and adults ≥ 15 years of age who were referred to the Department of Allergy for the first time and were suspected of allergy of any kind (not just inhalant or food allergy). Consecutive new patients were considered in order to avoid bias from any prior knowledge of the patient's clinical status. The investigators aimed at acquiring 20–30 positive samples per allergen from newly diagnosed allergic patients for the most prevalent allergens in the Breda-Oosterhout region. Patients were excluded if they were taking medication (e.g., antihistamines) that could interfere with the skin tests or if they had received immunotherapy during the previous year. The study's recruitment period ran from November 2001 to May 2002.

All participants gave informed, written consent for drawing an additional tube of blood at the time of the first visit for the purpose of the clinical evaluation study, i.e., for parallel testing on the IMMULITE 2000 and UniCAP 100.

Study design

The study design is presented in Figure 1. As shown in the left arm, routine diagnoses were assigned – and patient treatments instituted – by the allergist based on standard clinical practice, i.e., based on the integration of clinical history and examination, specific skin tests, and eventually challenge tests, and selected in vitro sIgE test results generated by the UNICAP 100. As shown in the right arm, study diagnoses were generated in a different manner for use as a gold standard in the clinical evaluation study. In brief, the study diagnoses were determined at the end of the study inclusion period by the same experienced allergist; however, in this case, because he was blinded to the routine diagnoses and to all in vitro diagnostic test results, the allergist's clinical judgment was based exclusively on the patient's history and clinical examination, combined with skin test results.

To this end, the allergist completed a separate case report form (CRF) designed for the clinical evaluation study – in addition to the regular medical records – and assigned a participant identifier code that was subsequently used for the additional blood sample. Each completed CRF included information, recorded during the first visit, on clinical symptoms, family history and medications. The CRFs were entered into a separate clinical database maintained by the Allergy Department. The allergist remained blinded to all in vitro diagnostic test results.

To ensure blinding at the technician level, the in vitro diagnostic tests were performed on coded fresh-frozen serum aliquots at different hospital locations, and results from the two assay systems were entered into separate laboratory databases, maintained at the Breda and Oosterhout sites, which were responsible for the IMMULITE 2000 and UniCAP 100 analyses, respectively.

The three databases were set up in Microsoft Access 97. The clinical and laboratory databases were linked to enable

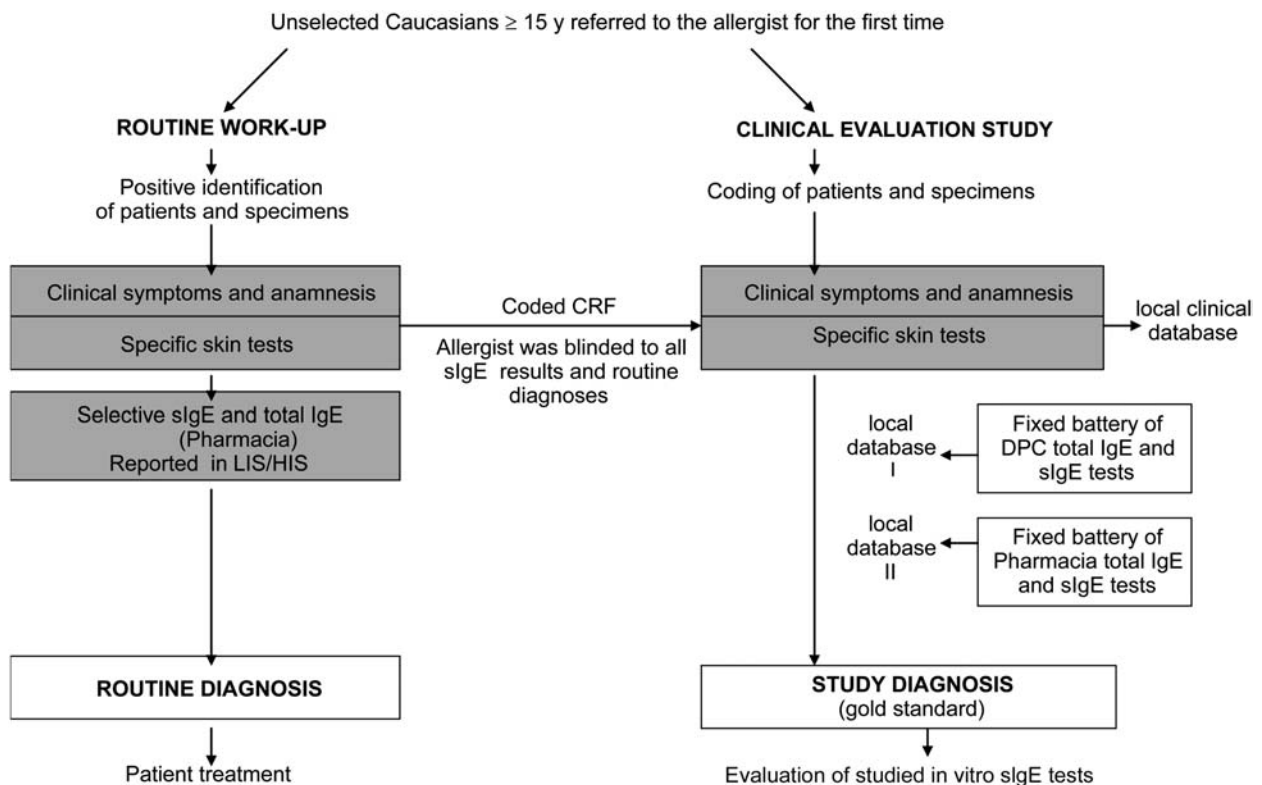


Figure 1 Study design used for the clinical evaluation study.

statistical calculations only after completion of all data collection for the study.

Routine patient work-up by the allergist

The allergist routinely performs a fixed battery of specific ICTs and SPTs, as listed below, to document sensitization in patients suspected of inhalant and/or food allergy. On an individual basis, additional *in vivo* testing (other skin tests, challenge tests, etc.) is performed as needed as part of the routine work-up. All of these *in vivo* test results are entered into the clinical database. In general, the allergist also requests blood tests for sIgE on an individual basis as part of the routine work-up; however, these *in vitro* results, performed on what was then our routine assay system, were not entered into the clinical or laboratory databases set up for the clinical study.

In all, 118 patients participated in the study. ICTs for inhalant allergens and SPTs for food allergens were performed on 108 and 105 of these patients, respectively. In accordance with good clinical practice, skin tests for inhalant and food allergens were not performed in 10 and 13 of the 118 patients included in the study, respectively, as there was no clinical necessity to perform such tests in these patients.

Individual allergens and allergen panels (mixes) used in the fixed battery of skin tests by the allergist and their associated codes were as follows:

- ICTs for grass panel 5: G3, orchard grass (*Dactylis glomerata*); G4, meadow fescue (*Festuca elatior*); G5, perennial rye grass (*Lolium perenne*); G6, Timothy grass (*Phleum pratense*); and G16, meadow foxtail grass (*Alopecurus pratensis*).
- ICTs for individual molds: M1, *Penicillium notatum*; M2, *Cladosporium herbarum*; M3, *Aspergillus fumigatus*; M6, *Alternaria alternata* (*A. tenuis*); and M7, *Botrytis cinerea*.
- ICTs for tree panel 3: T2, alder (*Alnus incana*); T3, birch (*Betula verrucosa*); and T4, hazel (*Corylus avellana*).
- ICTs for individual weeds: W6, mugwort (*Artemisia vulgaris*); and W9, English plantain (*Plantago lanceolata*).
- SPTs for individual foods: F1, egg white; F2, cow's milk; F3, codfish (*Gadus morhua*); F4, wheat (*Triticum aestivum*); F13, peanut (*Arachis hypogaea*); and F14, soybean (*Glycine max*).

Case definition

Because of their reliance on *in vitro* test results, the routine diagnoses (see Figure 1) were deemed inappropriate for evaluating the clinical performance of systems for assaying sIgE. Accordingly, study diagnoses, not based on any *in vitro* sIgE results, but solely on clinical history and symptoms and skin test results, were assigned. As we intended to focus on inhalant and food allergies in this clinical evaluation study, we defined patients as study diagnosis-positive (cases) and study diagnosis-negative (non-cases) as follows.

A study diagnosis-positive patient is a case with both (a) typical clinical symptoms of inhalant or food allergy, with a positive anamnesis or family history, and (b) positive skin tests for inhalant and/or food allergy. A study diagnosis-negative patient is any patient not fulfilling both of the criteria for a study diagnosis-positive patient. Thus, a study diagnosis-negative patient can be either (i) a true negative control – no clinical symptoms specific for inhalant, food or any other allergy whatsoever, negative anamnesis and family history, and negative skin tests for inhalant and food allergens – or (ii) a patient with an allergy other than inhalant or food allergy, or (iii) a patient belonging to the miscellaneous category (see Results).

In generating diagnoses for the study, patients presenting with asthma, rhinitis or atopic dermatitis and with at least one positive ICT for an inhalant allergen were classified as having an inhalant allergy. Patients with clinical symptoms suggestive of food allergy and with at least one positive SPT for a food allergen were classified as having a food allergy. Subjects with a positive skin test for an inhalant and/or food allergen who did not present with typical clinical symptoms of inhalant and/or food allergy were classified as asymptotically sensitized.

The study diagnoses assigned in this way were considered to be the gold standard for judging the performance of the IMMULITE 2000 Allergy System.

Clinical evaluation and diagnostic indices

Total agreement (TA), relative sensitivity and relative specificity were calculated as follows.

- Total agreement (%): (total number of results–number of discrepancies)×100/total number of results, where discrepancies are results positive by one method but negative by the other.
- Relative sensitivity (%): number of non-discrepant positives×100/number of positives by the reference method.
- Relative specificity (%): number of non-discrepant negatives×100/number of negatives by the reference method.

Confidence limits for differences (IMMULITE 2000 vs. UniCAP 100) in sensitivity, specificity or agreement were determined by the method of Newcombe and Altman, following current recommendations (17).

To evaluate the correspondence between skin test results in aggregate and the *in vitro* diagnostic inhalation screening and food allergy panel results, cumulative ICT and cumulative SPT were determined as follows.

Cumulative ICT takes into account the predefined individual intracutaneous tests for weeds and molds, as well as the tree and grass mixes tested for by the allergist. The subject's cumulative ICT was considered to be positive if at least one of the individual ICTs and/or mixes described in Routine patient work-up by the allergist was positive.

Cumulative SPT considers the individual skin prick tests performed by the allergist. The subject's cumulative SPT was considered to be positive if at least one of the individual skin prick tests mentioned in Routine patient work-up by the allergist was positive.

To evaluate the correspondence between skin test results and the *in vitro* diagnostic mold and weed panels, ICTs from the single molds and weeds presented in Routine patient work-up by the allergist were considered. Serological tree and grass panels were directly compared to the corresponding panels investigated by the allergist (see Routine patient work-up by the allergist).

Method comparisons

DPC and Pharmacia *in vitro* diagnostic results for single inhalant allergens and mixes are plotted in quantitative terms (kU/L). (Comparable scatterplots for the food allergens are not shown due to the limited number of patients with positive *in vitro* results for food allergens.)

Results

Study population

At the end of the 7-month period, the study population consisted of a total of 118 subjects. The overall

median age of the study population was 39 years [interquartile range (IQR)=27–51].

Of the subjects, 63 were classified as study diagnosis-positive on the basis of clinical symptoms and history and a positive skin test result for inhalation and/or food allergy; 55 were classified as study diagnosis-negative. Isolated inhalant allergy was diagnosed in 54 of the study diagnosis-positive subjects: allergic asthma ($n=17$) and/or allergic rhinitis ($n=44$) and/or allergic atopic dermatitis ($n=10$), the sum exceeding 54 as some patients had more than one clinical symptom. Isolated food allergy was demonstrated in seven study diagnosis-positive subjects. Combined inhalation and food allergy was present in two study diagnosis-positive cases.

The study diagnosis-negative group consisted of nine patients with allergies other than food or inhalation allergies, namely, insect venom allergy ($n=7$) and drug allergy ($n=2$); 24 patients were categorized as true negative controls and 22 were categorized as miscellaneous, having non-allergic rhinitis ($n=9$), urticaria/angio-edema ($n=3$), fatigue ($n=2$), non-allergic dermatitis ($n=2$), pruritis ($n=1$), anaphylaxis ($n=1$), bronchitis ($n=1$), and/or asymptomatic sensitizations ($n=12$).

Analytical performance of the in vitro diagnostic systems

During the clinical evaluation study, both analytical systems had stable performance and easily fulfilled the minimal performance targets for total imprecision of $\leq 15\%$ coefficient of variation for IgE antibody assays in the medium and high ranges, as recommended by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (18).

To document day-to-day precision, low- and high-level controls were analyzed throughout the clinical evaluation study in the same batches as the clinical specimens. Altogether, 18 analytical runs were performed during the clinical evaluation study. On the IMMULITE 2000, bilevel specific IgE controls (DPC catalog numbers L2UNC1 and L2UNC2) were used, with

total imprecision of 5.3% at 2.3 kU/L and 6.9% at 10.5 kU/L.

Performance of the inhalant screenings

AlaTOP and Phadiatop screening tests were positive in 81% and 83%, respectively, of the patients with clinical symptoms of inhalant allergy (Table 1). Compared to cumulative ICTs, AlaTOP detected 89% of the patients, whereas Phadiatop detected 96%. Compared to the study diagnosis, 91% of the patients were detected by AlaTOP as compared to 94% by Phadiatop. The AlaTOP ability to detect patients with clinical symptoms of inhalant allergy was similar to that of the cumulative ICTs performed by the allergist. The specificity of AlaTOP and Phadiatop relative to the study diagnosis was 75% and 72%, respectively. TA relative to the study diagnosis was the same for AlaTOP and Phadiatop (82%).

As mentioned above, relative to clinical symptoms (such as asthma, rhinitis and atopic dermatitis) suggestive of inhalant allergy, AlaTOP had a sensitivity of 81% and there appeared to be 12 false negatives. However, 10 out of the 12 had negative ICTs and were confirmed not to be suffering from inhalant allergy, reducing the number of false negatives to 2 out of 63. Moreover, 14 positive AlaTOP results were obtained on patients without clinical symptoms suggestive of inhalant allergy, but nine of these corresponded to positive ICTs and were thus categorized as being sensitized, diminishing the number of false-positive AlaTOPs to 5 out of 55.

Specificity estimates can best be derived from the true negative controls in the study diagnosis-negative subjects. In this group, specificity was 96% for both AlaTOP and Phadiatop, with inhalant screening negative in 23 out of 24 controls.

In 90% (62/69) of the Phadiatop-positive samples, AlaTOP was also positive. In 94% (46/49) of the Phadiatop-negative samples, AlaTOP was also negative. TA between Phadiatop and AlaTOP was 92%. Discrepant inhalant allergy screenings (10 out of 118) are summarized in Table 2. From this Table, it can be observed that AlaTOP and Phadiatop yielded false

Table 1 Correspondence of in vitro diagnostic inhalant allergy screenings to clinical symptoms, cumulative intracutaneous tests and study diagnosis of inhalant allergy.

Y vs. X	Total agreement, %	Relative sensitivity, %	Relative specificity, %
AlaTOP vs. CS	78.0 (92/118)	81.0 (51/63)	74.5 (41/55)
Phadiatop vs. CS	76.3 (90/118)	82.5 (52/63)	69.1 (38/55)
Cum. ICT vs. CS	78.8 (93/118)	82.5 (52/63)	74.5 (41/55)
AlaTOP vs. Cum. ICT	90.7 (98/108)	89.4 (59/66)	92.9 (39/42)
Phadiatop vs. Cum. ICT	94.4 (102/108)	95.5 (63/66)	92.9 (39/42)
AlaTOP vs. study Dx	82.2 (97/118)	90.7 (49/54) ^a	75.0 (48/64)
Phadiatop vs. study Dx	82.2 (97/118)	94.4 (51/54)	71.9 (46/64)

CS, characteristic clinical symptoms for inhalant allergy; Cum. ICT, cumulative intracutaneous tests for inhalant allergy. The cumulative ICT takes into account the predefined individual intracutaneous tests for weeds and molds, as well as the results of tree- and grass-mix assays performed by the allergist (see Routine patient work-up by the allergist). The cumulative ICT is considered positive if at least one of the individual ICTs and/or mixes as mentioned in Routine patient work-up by the allergist is positive. Dx, diagnosis of inhalant allergy. ^a AlaTOP (DPC) reported two more false negatives than Phadiatop (Pharmacia). Performances are presented as percentage (n).

Table 2 Discrepant inhalant allergy screenings (n = 10/118).

ID	DPC total IgE, kU/L	CS	AlaTOP (slgE: kU/L, class)	Phadiatop (slgE: kU/L, class)	ICT	Study diagnosis
81488	60	Yes	Neg (D1: 0.18, class 0)	Pos (D1: 0.55, class 1)	Pos (D1)	Allergic rhinitis
41402	328	No	Neg (no slgE)	Pos (no slgE)	N/A	No inhalant allergy
59245	111	No	Neg (no slgE)	Pos (Gx3: 0.52, class 1)	Neg	No inhalant allergy
29816	254	No	Neg (no slgE)	Pos (D1: 0.47, class 1)	Neg	No inhalant allergy
62763	75	Yes	Neg (no slgE)	Pos (E5: 1.35, class 2)	Pos (E5)	Atopic dermatitis
26972	443	No	Neg (E5: 0.20, class 0)	Pos (Wx3: 1.07, class 2)	Pos	Sensitization to weed
25726	489	No	Neg (no slgE)	Pos (D1: 1.58, class 2)	Pos	Sensitization to D1 and contact dermatitis
76710	1177	No	Pos (no slgE)	Neg (no slgE)	Neg	No inhalant allergy
46869	521	No	Pos (E5: 0.17, class 0)	Neg (no slgE)	Neg	Control subject
82459	120	Yes	Pos (Gp3: 0.23; Mp1: 0.20; E5: 0.12, class 0)	Neg (no slgE)	N/A	No inhalant allergy

CS, clinical symptoms characteristic for inhalant allergy; ICT, intracutaneous skin test for inhalant allergy; N/A, no available data.

Table 3 Comparison of single inhalation slgE tests to their corresponding intracutaneous tests.

Y vs. X	Total agreement, %	Relative sensitivity, %	Relative specificity, %
DPC			
D1 vs. ICT	93.5 (101/108)	87.5 (35/40)	97.1 (66/68)
E1 vs. ICT	94.4 (102/108)	89.7 (26/29)	96.2 (76/79)
E3 vs. ICT	96.3 (104/108)	81.8 (9/11)	97.9 (95/97)
E5 vs. ICT	79.6 (86/108)	35.5 (11/31)	97.4 (75/77)
Pharmacia			
D1 vs. ICT	90.7 (98/108)	90.0 (36/40)	91.2 (62/68)
E1 vs. ICT	91.7 (99/108)	75.9 (22/29)	97.5 (77/79)
E3 vs. ICT	94.4 (102/108)	72.7 (8/11)	96.9 (94/97)
E5 vs. ICT	87.0 (94/108)	67.7 (21/31)	94.8 (73/77)

Performances are presented as percentage (n).

Table 4 Comparison of inhalation mixtures to intracutaneous tests.

Y vs. X	Total agreement, %	Relative sensitivity, %	Relative specificity, %
DPC			
Tp9 vs. ICT ^a	90.7 (98/108)	73.5 (25/34)	98.6 (73/74)
Gp3 vs. ICT	96.3 (104/108)	95.0 (38/40)	97.1 (66/68)
Mp1 vs. ICT	83.3 (90/108)	36.0 (9/25) ^b	97.6 (81/83)
Wp3 vs. ICT	84.3 (91/108)	39.1 (9/23) ^c	96.5 (82/85)
Pharmacia			
Tx9 vs. ICT	90.7 (98/108)	76.5 (26/34)	97.3 (72/74)
Gx3 vs. ICT	96.3 (104/108)	95.0 (38/40)	97.1 (66/68)
Mx1 vs. ICT	83.3 (90/108)	36.0 (9/25) ^b	97.6 (81/83)
Wx3 vs. ICT	88.9 (96/108)	60.9 (14/23) ^c	96.5 (82/85)

Performances are presented as percentage (n). ^a ICT results are from the combined predefined individual mold and weed assays (see Routine patient work-up by the allergist). Serological tree and grass panels are directly compared to the corresponding panels investigated by the allergist (see Routine patient work-up by the allergist). ^b Identical patients are positive. ^c Difference is significant at $\alpha = 0.05$.

positives relative to study diagnosis in three out of the ten discrepant cases; that is, AlaTOP itemization in predefined slgEs indicated that slgE levels were either not detectable or ≤ 0.35 kU/L, whereas Phadiatop itemization revealed no demonstrable slgE in one out of three discrepant cases and minimal (class 1) reactivity in the two remaining cases. In addition, AlaTOP missed four allergic/sensitized patients compared to Phadiatop.

Performance of inhalant allergens and panels

The performance of the D1, E1, E3 and E5 serological tests compared to the corresponding ICTs is presented in Table 3. It can be observed that there was 82–90% correspondence of positive IMMULITE 2000 slgE test results with positive ICTs for D1, E1 and E3. As for E5, the DPC assay recognized only one out of three positive ICTs.

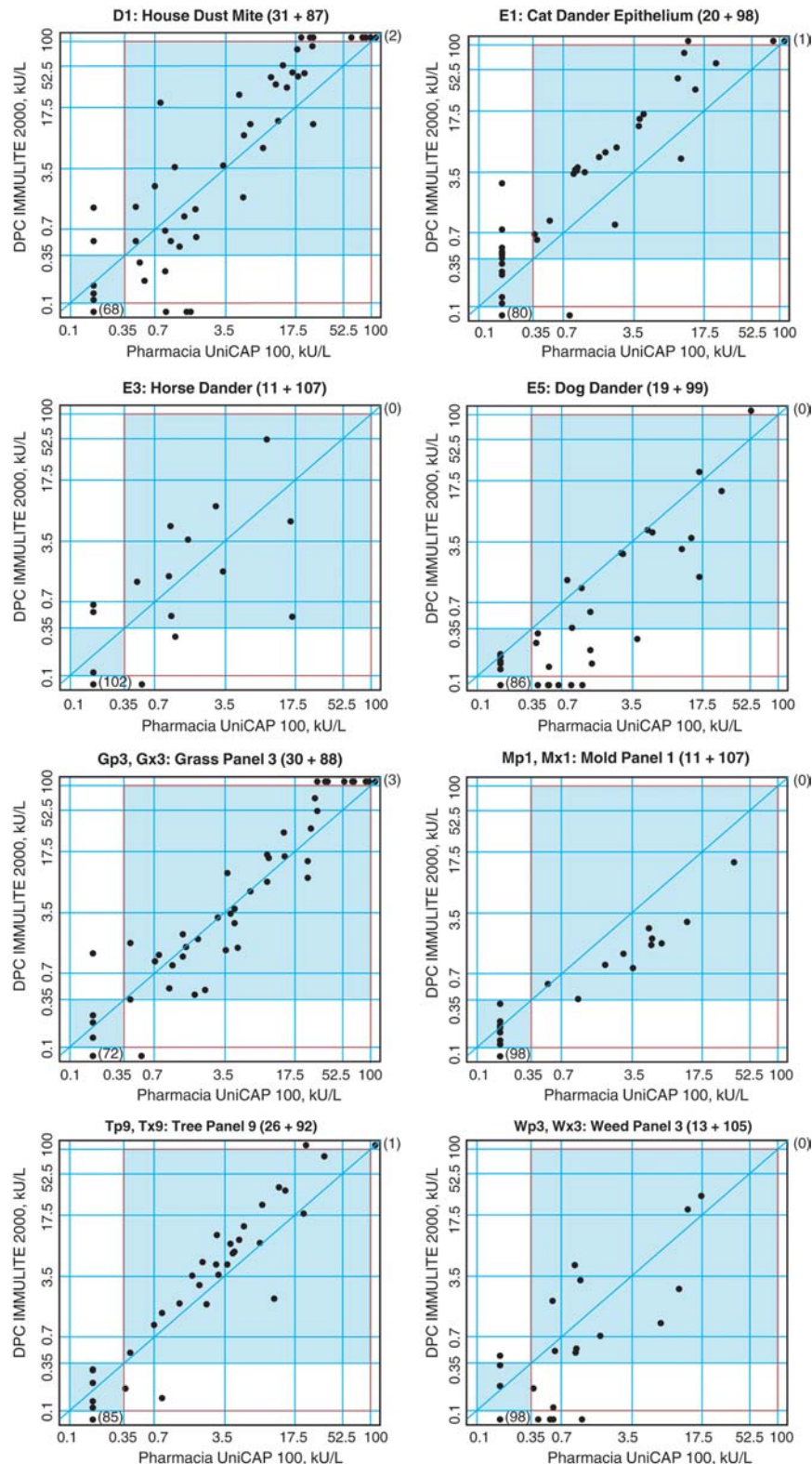


Figure 2 Scatterplots of DPC and Pharmacia single allergen assays and mixes with results expressed in quantitative terms (kU/L). The plotting areas are square and have the same limits on each axis; hence, the major diagonal represents the line of identity, which is highlighted to facilitate visual comparison of the assay results. The scatter plots use a logarithmic scale on both axes for concentration, and gridlines demarcate the implied class boundaries, which are essentially the same for both assays. The assays differ in their working ranges (0.35–100 kU/L for the UniCAP 100 and 0.10–100 kU/L for the IMMULITE 2000); these working ranges are indicated by a rectangular frame within the plotting area. Results with "explicit" numeric values by both methods plot within this framed region, whereas results outside the working range – i.e., results reportable simply as less than or greater than some value – by one method and/or the other are plotted at the visual center of the "gutter" surrounding this region. The number of results out-of-range-low by both methods and the number of results out-of-range-high by both methods are recorded parenthetically on the lower left and upper right of the plotting area, respectively. The scatter plot header indicates the number of mutually explicit results plus the number of results out-of-range high or low for that allergen.

Table 4 summarizes results for the tree, grass, mold and weed panels. For the weed panel, the sensitivity of the DPC assay relative to the skin test results was 39%, compared to 61% for Pharmacia, the difference was significant at $\alpha = 0.05$ (difference = 22%, CI 3–38%). For the other three panels, relative sensitivities for the two assay systems were comparable, ranging from 36% to 95%. The DPC and Pharmacia mold panels demonstrated equally disappointing sensitivity; this may be explained in part by their failure to recognize *Botrytis cinerea*, which was responsible for 6 out of 16 positive ICTs (data not shown).

A positive AlaTOP screen was corroborated by positive test results for single inhalant allergens and/or panels in 97% of the samples; a negative AlaTOP was corroborated by negative results for single inhalant allergens and panels in 94% of the samples. The corresponding figures for Phadiatop were 99% and 98%, respectively (data not shown).

Method comparisons for inhalant allergy

From Figure 2 it can be observed that there is fair correlation between the quantitative results of the two in vitro sIgE systems across the measuring range. In five out of eight scatterplots, the data are more or less randomly scattered around the line of identity, whereas in the remaining three scatterplots, the majority of the data points are either above the line of identity or below it. In the case of E1, for example, the DPC assay appears to be more reactive than the Pharmacia assay, while the opposite is true for E5 and for the mold panel: in these panels, nearly all points lie below the line of identity.

Performance of food screening

The number of patients included with food allergies was very limited ($n=9$). Table 5 shows that on both platforms, food panels (Fp5, Fx5) were positive in less than half of the patients with clinical symptoms of food allergy. Compared to cumulative SPTs, the DPC Fp5 and Pharmacia Fx5 detected 8 and 9, respectively, out of 14 SPT-positive patients. Compared to the study diagnosis, six and seven out of nine patients with food allergy were detected by the DPC and Pharmacia food panels, respectively. In particular, the DPC system missed two food allergies for F13 and one

food allergy for F17 and other nuts, out of nine food allergies in the study diagnosis-positive patients (data not shown).

From Table 5 it is clear that the specificity of the DPC Fp5 assay relative to clinical symptoms, cumulative SPTs and study diagnosis is 96–100%, compared to 93–97% for Pharmacia. By reference to the study diagnosis of “no food allergy”, discrepant food allergy screenings were found in 5 out of 109 study diagnosis-negative patients. These discrepancies are apparently due to false-positive Pharmacia Fx5 results – no detectable sIgE in three patients, measurable F4 and F13 in one patient, and measurable F4 and F14 in another patient – leading to lower relative specificity for the Pharmacia food allergy screening (94%) compared to DPC (98%). When specificity estimates are derived from the true negative controls (a subset of the study diagnosis-negative patients), specificity for both Fp5 and Fx5 was 100%.

Performance of food allergens

Comparison of single food sIgE tests to SPTs reveals that negative sIgE test results for F1, F2, F3, F4, F13 and F14 correspond with negative SPTs in 95–100% of the patients for DPC and in 89–99% of the patients for Pharmacia. The sensitivity data are inconclusive, however, due to the limited number of patients with food allergies enrolled in the study.

Discussion

In this prospective clinical study on the performance of the IMMULITE 2000 Allergy System, the study population consisted of unselected patients who were referred to the allergist for the first time. Consequently, the population studied reflects the patient population in routine clinical practice. Unselected patient inclusion is often used (19), but contrasts with the selected inclusion approach of, e.g., Ownby et al. (20). Nevertheless, this study has three major strengths. Firstly, the performance of the DPC IMMULITE 2000 Allergy System was evaluated against a gold standard consisting of clinical diagnoses based solely on characteristic clinical symptoms, history and skin test results, and entirely independent of any in vitro sIgE

Table 5 Clinical performance of food allergy screenings.

Y vs. X	Total agreement, %	Relative sensitivity, %	Relative specificity, %
Fp5 vs. CS	89.8 (106/118)	33.3 (4/12)	96.2 (102/106)
Fx5 vs. CS	87.3 (103/118)	41.7 (5/12)	92.5 (98/106)
Cum. SPT vs. CS	88.1 (104/118)	50.0 (6/12)	92.5 (98/106)
Fp5 vs. Cum. SPT	94.3 (99/105)	57.1 (8/14)	100.0 (91/91)
Fx5 vs. Cum. SPT	92.4 (97/105)	64.3 (9/14)	96.7 (88/91)
Fp5 vs. Study Dx	95.8 (113/118)	66.7 (6/9) ^a	98.2 (107/109)
Fx5 vs. Study Dx	91.5 (108/118)	77.8 (7/9) ^a	93.6 (102/109)

Performances are presented as percentage (n). Fp5, food allergy screening from DPC; Fx5, food allergy screening from Pharmacia. CS, characteristic clinical symptoms for inhalant allergy; Cum. SPT, cumulative skin prick test. The results from the F1, F2, F3, F4, F13 and F14 skin prick tests are combined to determine the cumulative SPT. The cumulative SPT is positive when at least one of the individual SPTs is positive. Dx, study diagnosis. ^a Identical patients were detected.

test results. Secondly, inter-observer variability was excluded, as the study diagnoses were all assigned by a single experienced allergist. Thirdly, selection bias and (in the case of Pharmacia system) circularity were minimized by including only new (first visit) unselected patients referred to the allergy clinic and by imposing multiple levels of blinding, both at the level of the allergist and at the level of the laboratory technicians. A principal limitation of this study is the fact that the sensitivity data for food allergen results are inconclusive, due to the low number of patients with food allergies involved in the study. Hence, additional clinical studies focusing on the diagnostic performance of IMMULITE 2000 assays for food allergens are needed.

Overall, the study results are consistent with those of other investigators who have evaluated newer technologies for the in vitro measurement of allergen-specific IgE (3, 19, 20). When focusing on inhalant allergy (Table 1), the diagnostic sensitivity indices calculated for the inhalant screening show that the DPC AlaTOP compares well with characteristic clinical symptoms, in vivo skin test results and study diagnoses based on an integration of clinical symptoms, history and skin test results in 75–90% of the study diagnosis-positive patients. The diagnostic specificity for the DPC AlaTOP screening compared to study diagnoses is apparently low (75%), due to the fact that the study diagnosis-negative group encompassed not only true negative controls (no clinical symptoms, negative anamnesis and negative skin tests), but also patients both with allergies other than inhalant allergy and a miscellaneous group with, e.g., asymptotically sensitized patients. It is known that the presence of, e.g., asymptotically sensitized individuals results in falsely low estimates of test specificity (20). Our specificity data are consistent with those reported by others (3, 19, 20). As expected, the diagnostic specificity derived from the negative control group is high (96%). From the screening data it can be concluded that the performance of the AlaTOP inhalant screen is comparable to that of Phadiatop.

With exceptions for E5 and mold and weed panels (Tables 3 and 4), the relative sensitivity of the DPC inhalation allergens and panels compared to in vivo skin test results is 74–95%. This is comparable to what others have observed (3, 19, 20) and to the performance of the UNICAP 100 system from Pharmacia in this study. For the IMMULITE 2000 Allergy system, the E5 and weed panel assays match with the skin test results in less than 40% of the ICT-positive patients. For the purpose of our internal decision-making process with respect to the eventual purchase of IMMULITE 2000 systems, another E5 lot (lot 112) was tested after the clinical evaluation study was finished. This lot showed a nearly two-fold improvement in relative sensitivity relative to ICT (67% instead of 35.5%; data not shown). Routinely, E5 extracts from DPC originate from a mix of different dog breeds. The extract used for lot 112 had the same origin as for the lot tested during the clinical evaluation study, but it was from a different raw material lot and was extracted in a

slightly different manner – in particular, the weight/volume ratio of extraction buffer to protein was modified (personal communication from DPC).

Notwithstanding that it has the same allergen composition, the IMMULITE 2000 weed panel is apparently less sensitive than the Pharmacia panel (Table 4 and Figure 2). In general, it is obvious that the performance of IgE antibody assays depends on the quality of the allergens and on their availability to the in vitro reaction site. The DPC specific allergen assays with biotinylated allergen-polymers in the liquid phase should at least have the theoretical advantage of minimal steric hindrance because of the intact three-dimensional structure of the allergens. However, intermethod discrepancies must be expected because allergen preparations are currently not sufficiently standardized, i.e., no recombinant forms exist as yet (7).

Mold panel performance poses identical sensitivity problems for both sIgE assay systems (Table 4). We suggest that both manufacturers consider adding *Botrytis cinerea*, as this allergen accounted for 6 out of 16 missed mold-positive ICTs. After all, it has a worldwide distribution, occurring mainly in humid, temperate and subtropical regions. *Botrytis cinerea* is found regularly in the soil, although its proportion of the total fungus population is not high. It can be parasitic on a wide range of plants, causing blight or rot of leaves, flowers and fruits; it is called gray mold, as it covers decayed tissues with conidiophores (e.g., gray mold of cabbage, lettuce or tomato). It is especially observed in connection with soft fruits, such as strawberries and grapes. Positive skin test reactions have been observed in several mold-sensitive patients, indicating its importance as a mold allergen. In Sweden, 1250 patients having allergy-related complaints were skin prick tested with *Botrytis cinerea*. This induced a strong reaction in 50% of the mold-sensitized patients (21). According to Karlsson-Borga and co-authors, IgE antibodies against *Botrytis* are common (22), accounting for a frequency rate of positive tests second to *Cladosporium* in a group of samples collected in Scandinavia. In another set of samples collected in the USA, *Botrytis* accounted for the greatest frequency of positive tests, whereas *Alternaria*, often referred to as the most important mold to consider, had a much lower frequency. Kauffman and coworkers (23) studied the allergenic properties of *Botrytis cinerea*, finding IgE-binding components in both early- and late-phase antigens.

Food screening via Fp5 on the IMMULITE 2000 exhibited low sensitivity relative to clinical symptoms, SPTs and study diagnoses in this limited sample (Table 5). This was also true for screening via Fx5 on the UniCAP 100. False negatives generated by the DPC Fp5 food screen can be expected for food allergies to F17 (and other nuts as well) because the F17 allergen is not included in the food screening panel. Missing food allergies to F13 can be expected in the case of, e.g., lability of major allergens in food extracts (7). The DPC Fp5 screen did not yield any false positives in the patients studied due to the high

specificity of the Fp5 panel in both the study diagnosis-negative group (96–100%) and the negative control group (100%).

We conclude that the diagnostic accuracy of the IMMULITE 2000 screens, panels and specific allergens for inhalant and food allergy is similar to that of the corresponding tests on the UniCAP 100. This makes the DPC IMMULITE 2000 Allergy a viable and interesting alternative to second-generation systems currently available. Further optimization of certain IMMULITE 2000 allergens is certainly needed, as demonstrated in this study for E5, and the weed and mold panels. On balance, however, given comparability in terms of clinical performance, the choice of systems for measuring sIgE can be made on other grounds, such as ease of use, automation, speed, versatility and the possibility of consolidation with endocrinology tests and tumor markers.

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