

A clinical evaluation in children of the Pharmacia ImmunoCAP system for inhalant allergens

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Summary

Background The Pharmacia ImmunoCAP system (CAP) for assaying serum IgE specific antibodies was evaluated in a clinical setting against skin-prick test (SPT) performed using Dome/Hollister-Steir allergen extracts. The five common inhalant allergens *D. pteronyssinus*, *D. farinae*, mould mix, grass mix and cat epithelium were tested concurrently by both methods in 167 children aged 7.5–12 years. The specific SPT for *D. pteronyssinus* and *D. farinae* were also tested against the CAP house dust mite (HDM) mix.

Objective The purpose of the study was to determine the sensitivity and specificity of the Pharmacia ImmunoCAP system for detecting serum IgE specific antibodies to inhalant allergens in a clinical setting, using the SPT result as the 'gold standard'.

Methods The SPTs were performed using Dome/Hollister-Steir allergen extracts. The serum IgE specific antibodies were quantitated using the radioimmunoassay version of the Pharmacia ImmunoCAP system. A history of allergic disease was assessed using a validated questionnaire.

Results SPT gave more positive reactions than CAP with the exception of cat epithelium. The concordance between SPT and CAP results was 91% over all the tests. The concordance with SPT was slightly higher for the specific CAP for *D. pteronyssinus* and *D. farinae* (93% and 95% respectively) than for the CAP HDM mix (86% and 90% respectively). There was a higher proportion of positive results for both SPT and CAP in the 115 children defined as having a history of allergic disease. Using SPT defined allergy as the gold standard, the sensitivity of the CAP system was 87% for the two specific house dust mites but was lower for cat epithelium (67%), mould mix (59%) and grass mix (46%). The sensitivity of the CAP system improved for *D. pteronyssinus* (96%) and the HDM mix (91%) when tested in subjects defined as having a history of allergy associated disease. The specificity of the CAP system showed less variation between allergens and ranged from 90–99%.

Conclusion The results of this study of children aged 7.5–12 years demonstrate that, for the inhalent allergens tested, the Pharmacia ImmunoCAP system performs well in the setting of known allergic disease.

Keywords: ImmunoCAP, inhalant allergens, skin prick, allergic disease, children

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Introduction

Assessment of atopic status is appropriate in the investigation of respiratory diseases such as rhinitis or asthma; in anaphylactic diseases resulting in urticaria, angioedema or gastrointestinal reactions; and where atopy is suspected as the cause of dermatitis or gastroenteropathy. When a diagnosis of allergy is suspected, the detection of allergen specific IgE antibodies can be determined by *in vivo* skin-prick testing, or a variety of *in vitro* assays. Correctly performed skin-prick tests have retained a reputation as the most practical and valuable test for the detection of allergen-specific IgE. However, in some circumstances it may not be possible to perform SPT, such as a history of anaphylaxis, skin disorder, interfering medication or a non-compliant patient. While positive tests for allergen specific IgE do not diagnose allergy they provide supportive evidence in an appropriate clinical setting.

Until recently, the *in vitro* detection of allergen-specific IgE has utilized either a paper-disc radioallergosorbent test (RAST) or a variety of other solid phase binding systems [1,2]. The RAST is the most widely utilized methodology and has been shown to have excellent specificity [90–98%] but relatively low sensitivity (60–70%) when compared with SPT [3,4]. In order to increase the sensitivity of detection of IgE specific allergens the RAST has been replaced with a new solid phase capture system (CAP) which can bind up to three times more antibodies than the corresponding paper disc [5].

The Pharmacia ImmunoCAP system has been evaluated against the Phadebas RAST and SPT [4–7]. When compared with SPT as the 'gold standard', the CAP system has been shown to have greater sensitivity [80–95%] than the RAST and to have similar specificity [85–98%]. The published studies of the Pharmacia ImmunoCAP system have compared the SPT results with standardized Pharmacia allergen extracts [4,7], standardized extracts from Stallergènes Laboratories [6] and non-standardized extracts from Bencard [3]. There are no comparative data for the Dome/Hollister-Steir SPT results and the Pharmacia ImmunoCAP system.

This study evaluated the Pharmacia ImmunoCAP system against the Dome/Hollister-Steir SPT in a cohort of children participating in a research programme investigating the incidence and determinants of asthma and allergy in the general community. The CAP allergen results were compared with the SPT result and related to a clinical diagnosis of allergic disease.

Methods

Subjects

The study population consisted of 167 children who were participating in a research programme investigating the incidence and determinants of asthma and allergy in the

general community. The children had been recruited at birth into a prospective study of the development of mucosal immunity [8]. The children, aged 7.5–12 years, were assessed for atopy by a previously validated questionnaire [9] and classified as having allergy associated disease if they had a history of hay fever, eczema, or asthma, based on the parent-completed questionnaire. A classification of asthma was based on questions asking whether the child had ever wheezed, had wheezed in the last 12 months, wheezed during exercise, coughed at night, used medications for asthma or had been diagnosed as having asthma by a doctor. Allergic rhinitis and atopic eczema classifications were based on questions asking whether the child had ever had hay fever or eczema.

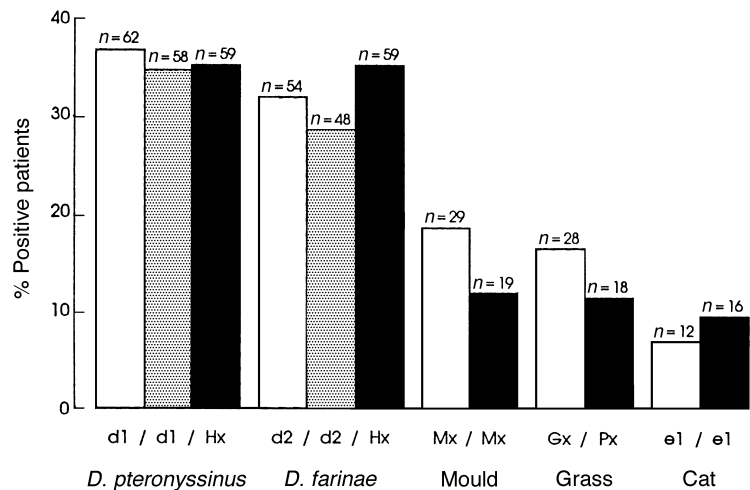
Skin-prick tests

Skin-prick tests were performed by four members of the research team in a standardized procedure. The tests were done on the forearm according to the method of van Asperen *et al.* [10] with standardized allergen extracts supplied by Dome/Hollister-Steir (Bayer Pharmaceutical, Australia) for two house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), mould mix (*Alternaria*, *Aspergillus* mix, *Hormodendrum*, *Penicillium* mix), mixed grasses (kentucky blue, meadow fescue, orchard, perennial rye, redtop, sweet vernal, timothy), cat fur (cat hair and epithelium) and positive (histamine) and negative (glycerine) controls. A mean weal diameter of 3 mm or greater at 15 min was considered positive. Eleven subjects were not tested by mould mix.

CAP allergens

Blood was collected on the same occasion as the SPT. The sera were stored in aliquots at -70°C . Thawed samples were tested in large batches. Samples were not refrozen and additional aliquots were thawed if necessary. Undiluted sera were tested using the radioimmunoassay version of the Pharmacia ImmunoCAP System (Kabi Pharmacia, Sweden) for IgE specific antibodies to *Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), house dust mix (hx2: Hollister-Steir laboratories house dust, *D. pteronyssinus*, *D. farinae* and *Blattella germanica* [cockroach]), mould mix (mx2: *Penicillium notatum*, *Cladosporium herbarium*, *Aspergillus fumigates*, *Candida albicans*, *Alternaria alternata*, *Helminthosporium halodes*), Australian pollen mix (tx7: *Olea europaea*, *Salix caprea*, *Pinus strobus*, *Eucalyptus* spp, *Acacia longifolia* and *Melaleuca leucadendron*) and cat dander (e1). The specific IgE assays were calibrated against the second international reference preparation WHO standard 70/502 for IgE in the range

Fig. 1. Number and percentage of subjects with positive results for skin-prick test (SPT) or Pharmacia CAP system (CAP) for specific allergens or group mixes for the total study population ($n = 167$). □. SPT. ■. CAP allergen mix. ▨. CAP-specific allergen.



0.35–100 kU/L. The positive cut-off limit was ≥ 0.35 kU/L.

Statistics

SPT was used as a gold standard in the analysis. Patients were classified as positive or negative for each test according to the previously stated criteria. Confidence intervals for sensitivity and specificity were calculated using the exact binomial distribution for $n \leq 100$ and using the normal approximation for $n > 100$. Spearman's rank correlation coefficient was used to describe the relationship between the mean diameter of the SPT weal and the CAP level in those patients positive for both tests.

Results

History of allergy

Of the 167 subjects, 115 were classified as having a history of allergy associated disease based on a history of one or more of asthma ($n = 65$), wheeze ($n = 68$), hay fever ($n = 61$) and eczema ($n = 54$). Eleven subjects were not tested by SPT for mould of whom seven were classified in the group with a history of allergic disease.

Prevalence of positive results for SPT and CAP system

The prevalence of SPT positive atopy in the overall population ranged from 37% for HDM to 7% for cat epithelium (Fig. 1). The concordance between SPT and CAP was 91% overall for all allergens tested (Table 1). The discordant results favoured positive SPT results with the exception of the comparisons for cat epithelium and the comparison of the CAP house dust mix with the *D. farinae* SPT result (Fig. 1). There was a higher proportion of positive SPT results for each

allergen in those defined as having a history of allergy associated disease (Fig. 2).

Correlation between SPT and CAP system

To explore whether there were quantitative associations between the size of the SPT and the level of CAP-specific IgE, Spearman rank correlation coefficients were calculated for those subjects with positive results to both SPT (mean weal diameter) and serum CAP allergen (>0.35 kU/L) (Table 2). Significant correlations were observed between SPT *D. pteronyssinus* and both CAP *D. pteronyssinus* and CAP house dust mite mix, as well as SPT *D. farinae* and CAP house dust mite mix.

Sensitivity and specificity

The sensitivity of the CAP system was high for the house dust mite comparisons (range 79–89%) but much lower for the other allergens tested (Table 1). The sensitivity of the CAP system for *D. pteronyssinus* and cat epithelium was higher in the subjects defined as having a history of allergy associated disease than for the total study population (Table 3). The small sample size did not allow accurate estimates of sensitivity and specificity for the allergens with low prevalence, such as cat epithelium.

The specificity of the CAP system for the total study population varied for each specific allergen (Table 1). The specificity was similar for each allergen when tested in the subjects defined as having a history of allergic disease or no history of allergy-associated disease (Table 3).

Discussion

Consistent with previous investigations comparing the Pharmacia ImmunoCAP system with SPT [3,4,7] this study

Table 1. Comparison of results obtained by skin-prick test (S) and CAP system (C) for the overall study population ($n = 167$)

SPT Allergen	CAP Allergen	Concordant results			Discordant results			Sensitivity* % (95% CI)	Specificity* % (95% CI)
		S ⁺ /C ⁺	S ⁻ /C ⁻	Total no (%)	S ⁺ /C ⁻	S ⁻ /C ⁺	Total no (%)		
<i>D. pteronyssinus</i>	<i>D. pteronyssinus</i> (d1)	54	101	155 (93)	8	4	12 (7)	87.1 (76–94)	96.2 (94–98)
<i>D. pteronyssinus</i>	House Dust mix (HX2)	49	95	144 (86)	13	10	23 (14)	79.0 (67–88)	90.5 (88–93)
<i>D. farinae</i>	<i>D. farinae</i> (d2)	47	112	159 (95)	7	1	8 (5)	87.0 (75–95)	99.1 (98–100)
<i>D. farinae</i>	House Dust mix (HX2)	48	102	150 (90)	6	11	17 (10)	88.9 (77–96)	90.3 (88–93)
Mould mix**	Mould mix (MX1)	17	125	142 (91)	12	2	14 (9)	58.6 (39–76)	98.4 (97–100)
Grass mix	Pollen mix (APX2)	13	134	147 (88)	15	5	20 (12)	46.4 (28–66)	96.4 (95–98)
Cat epithelium	Cat epithelium (e1)	8	147	155 (93)	4	8	12 (7)	66.7 (35–90)	94.8 (93–97)

*Using SPT as the gold standard or (95% CI). ** Eleven subjects were not tested by SPT for mould ($n = 156$).

found that the prevalence of positive SPT results using Dome/Hollister-Steir extracts was higher than for the CAP allergen-specific IgE results, with the exception of cat epithelium. Overall, the level of concordance between SPT and CAP was high. As in previous studies [3,4] the discordant results favoured a positive SPT and negative CAP (6%), again with the exception of cat epithelium.

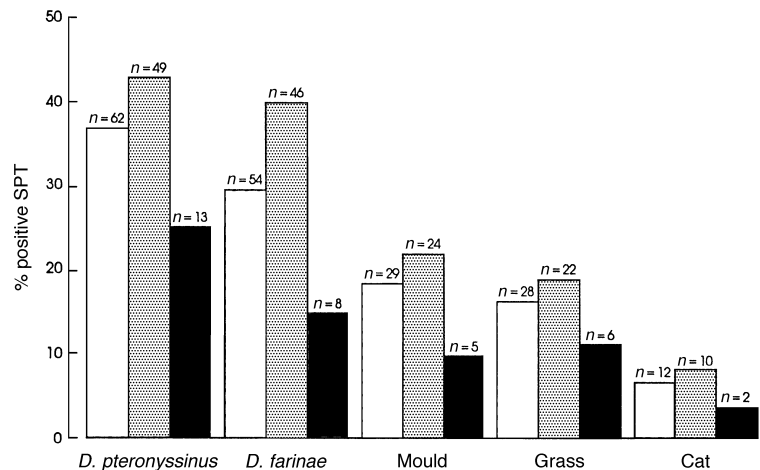
The sensitivity of the CAP system compared with SPT ranged from 79 to 89% for house dust mites in the overall population but improved substantially when evaluated in the children with a previous history of allergy associated disease (87–96%). The sensitivity of the CAP system for HDM was low in the children with no history of allergy (46–75%). The differences in sensitivity between children with or without a history of allergy associated disease were more obvious for the evaluation of *D. pteronyssinus*, which is the common house dust mite on the east coast of Australia [11–12], than for *D. farinae*. The sensitivity of the CAP system was much lower for the other allergen comparisons (46–67%) and did not improve significantly when the population was divided on the basis of a history of allergy associated disease.

The results of our study suggest that the Pharmacia ImmunoCAP system may be more sensitive for IgE-specific cat epithelium than SPT performed at the standard strength of extract used for SPT. Leimgruber *et al.* [4] have previously investigated a series of subjects with negative SPT and positive CAP for cat epithelium by crossed radioimmuno-electrophoresis and concluded that the CAP positive results were true positives. The composition of the cat extracts may account for the discordant results as this finding has not been seen in a similar study using different allergen extracts [13]. This raises doubts on the continued use of SPT as the 'gold standard' for detection of IgE antibodies to cat epithelium. The use of SPT as the 'gold standard' for other allergens requires further evaluation.

The specificity of the CAP system compared with SPT was high for all allergens tested and ranged from 90 to 99%. The specificity was higher for the individual specific house dust mite CAP results (96–99%) than for the house dust mite mix (90–91%), which contains a mixture of *D. pteronyssinus*, *D. farinae* and cockroach. In a clinical setting of suspected house dust mite allergy, where intervention is contemplated, it may be more appropriate to test for the specific house dust mite(s) than to screen with the HDMX.

Comparison of this study with previously reported evaluations of the CAP system indicated that the CAP system performed well when compared with the SPT results using Dome/Hollister-Steir allergen extracts. The sensitivities and specificities in this study were higher than those previously reported for comparisons with Bencard SPT allergens [3] and Pharmacia SPT allergens [7] for the

Fig. 2 Number and percentage of subjects with positive skin-prick test in the overall population and the subgroups with a history of allergic disease or no allergic disease. □. Overall population. ▨. Allergic disease. ■. No-allergic disease.



same CAP allergens and had similar specificities to those reported by Bousquet *et al.* [6] who used Stallergènes SPT allergens. This study had marginally lower sensitivities than those reported by Bousquet *et al.* [6]. The higher sensitivity in the Bousquet *et al.* study may have resulted

from their choice of study population, which assessed only subjects with respiratory symptoms at the time of their investigations. There was no selection bias of children exhibiting allergic diseases or associated respiratory symptoms in the current study.

Table 2. Spearman rank correlation coefficient (r_s) between SPT mean weal size and CAP allergen IgE levels in children positive for both tests (S^+/C^+ , Table 1)

SPT Allergen	CAP Allergen	n	Correlation coefficient (r_s)	Significance level (P)
<i>D. pteronyssinus</i>	<i>D. pteronyssinus</i>	54	0.49	0.0003
<i>D. pteronyssinus</i>	House dust mix	49	0.49	0.0016
<i>D. farinae</i>	<i>D. farinae</i>	47	0.15	0.28
<i>D. farinae</i>	House dust mix	48	0.28	0.05
Mould mix	Mould mix	17	0.20	0.50
Grass mix	Pollen mix	13	0.39	0.17
Cat epithelium	Cat epithelium	8	0.24	0.44

Table 3 Sensitivity and specificity of CAP allergen tests compared with SPT (as 'gold standard') for those with a history of allergic disease (n = 115) and those with no evidence of allergic disease (n = 52)

SPT Allergen	CAP Allergen	Allergic disease		No allergic disease	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
<i>D. pteronyssinus</i>	<i>D. pteronyssinus</i>	95.9	95.5	53.9	97.4
<i>D. pteronyssinus</i>	House dust mix	87.8	89.4	46.2	92.3
<i>D. farinae</i>	<i>D. farinae</i>	87.0	100	87.5	97.7
<i>D. farinae</i>	House dust mix	91.3	88.4	75.0	93.2
Mould mix	Mould mix	58.3	97.6	60.0	100
Grass mix	Pollen mix	45.5	94.6	50.0	100
Cat epithelium	Cat epithelium	70.0	93.3	50.0	98.0

Of the 11 subjects who were not tested by SPT for mould, seven were classified as having a history of allergic disease (n = 108).

The size of the SPT weal has been reported to be correlated with the severity of atopic disease [14]. The study by Pastorello *et al.* [7] of 15 allergens, reported a correlation between the size of the SPT weal and the CAP result for *D. pteronyssinus* and grass pollens only. The current study has shown that, with the exception of *D. pteronyssinus*, there was no quantitative correlation between the size of the SPT weal and the level of IgE-specific CAP antibody. The poor correlation may reflect differences in the antigenic composition of the Dome/Hollister-Steir SPT and Pharmacia CAP allergens, but most likely reflects the underlying differences in the biological mechanisms of SPT inflammation and IgE antibody production in serum.

The results of this study demonstrate that the Pharmacia ImmunoCAP system for the detection of IgE-specific antibodies performs well compared with the Dome/Hollister-Steir SPT results in the setting of allergic disease. However, the results indicate that the CAP allergen test is not an appropriate population screening test for atopy. The results also indicate that the ImmunoCAP system was superior to SPT for the detection of IgE-specific antibodies to cat epithelium, and in some clinical settings where the CAP system is preferred to SPT, it may be more appropriate to test for specific HDM rather than screen with the HDMX. The ImmunoCAP system has the capacity for reporting quantitative IgE-specific CAP levels which may be useful for monitoring desensitization therapy. In addition, the extensive range of allergens available for CAP testing make it useful in defined clinical situations where SPT allergens are not available or SPT is unsuitable.

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