

RAST in the Diagnosis of Dog Dander Allergy

A Comparison between Three Allergen Preparations Using Two Variants of RAST

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The results of the skin, bronchial or conjunctival provocation tests and of the radioallergo-sorbent test (RAST) with dog dander and hair extract preparations from two different producers were compared. 202 asthmatic children were included in the study. The clinical tests, Al-RAST and paper RAST, were performed using only one allergen preparation (Hu 073), while two allergen preparations (e2 and e5) were used in Phadebas® RAST.

A good correlation was generally found between the results of the clinical diagnostic tests, the clinical history and the results of RAST.

Phadebas RAST e2 was the most specific but the least sensitive: it gave a class 0 result in 18% and class 3-4 in 34.5% of the subjects with a positive provocation test. The corresponding numbers for e5 RAST were 6.0% and 70.2%, respectively. Al-RAST was equally sensitive, but had lower specificity than Phadebas RAST e5. The correlation between Phadebas RAST e5 and paper RAST Hu 073 was excellent, which indicates a remarkable similarity between these allergen extracts.

Using sensitive, immunochemically standardized Phadebas RAST e5 or Al-RAST methods, classes 0-1 can be regarded as negative, class 2 as doubtful and classes 3-4 as clinically positive results.

Key words: asthma; dog allergens; provocation test; RAST; skin test.

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CLINICAL ASPECTS

The Phadebas® RAST system using two allergen extracts, dog epithelium (e2) and dog dandruff (e5) was compared with a recently introduced RAST method, Al-RAST, using dog dander and hair as an allergen extract, in a study including 202 asthmatic children. An allergy score combining the clinical history, the skin prick test and the provocation test was used to determine the specificity and sensitivity of the different RAST systems.

Even though a good correlation was generally observed between the clinical tests and RAST, some important differences were demonstrated between the different RAST systems. The dog epithelium (e2) extract was found to be too insensitive to exclude dog allergy. Al-RAST, on the other hand, was less specific, i.e. it often gave class 2-3 results in subjects without other clear-cut evidence of dog allergy. Phadebas RAST with dog dandruff (e5) extract and Al-RAST were found to be useful for clinical purposes: a class 0-1 result ruled out the cases without dog allergy and a class 3-4 result was mostly associated with an apparent dog allergy. However, by these methods a RAST result class 2 was found to be doubtful and should be confirmed using other diagnostic criteria.

The diagnosis of allergy to dog dander and hair has been complicated by the varying quality of the different allergen preparations. Poor allergen preparations lacking proper biological and immunochemical standardization can lead to errors both in the *in vivo* and *in vitro* tests. In this regard, the most common *in vitro* test, RAST, is no exception.

The recent advance in the purification and characterization of dog dander and hair extracts (4, 5, 12) has provided allergists with new, immunochemically characterized allergen preparations, which have been introduced in routine diagnostic practice (16).

Allergen extracts adsorbed to aluminium hydroxide are widely used in the immunotherapy of allergic patients. Recently, allergens have also been coupled to aluminium hydroxide for diagnostic purposes, as a new modification of RAST, Al-RAST (19). Thus it is possible to use the same reagent, allergen extract adsorbed to aluminium hydroxide, for the *in vitro* diagnosis of allergy and for specific immunotherapy. However, there are no data available about the specificity and sensitivity of this new modified RAST in the diagnosis of specific allergy.

The purpose of the present study was to compare the RAST results obtained by using three different commercial allergen preparations as allergosorbent and to evaluate the usefulness of the Al-RAST compared with the ordinary paper RAST in the diagnosis of dog dander allergy. The RAST results were compared with the *in vivo* tests: the skin prick test and the provocation test, which were performed on all of the subjects.

MATERIAL AND METHODS

Patients

202 children from the Children's Asthma Register of the Pediatric Department of the University of Turku (10) were included in the study. The mean age of the children was 10.4 years (range 4–15 years). 122 of the patients (60%) were males. None of the patients had previously been hyposensitized with dog or cat allergen extracts. Seventeen of them had previously received hyposensitization therapy with house dust and/or mite allergen extracts. Their clinical history was recorded by means of a detailed questionnaire. The children were divided into three groups according to the clinical history of allergy to dogs: a) negative, b) doubtful and c) positive.

Allergen extracts

The allergen extract for the skin prick test (in the following denoted Hu 073) was obtained from the Allergologisk Laboratorium A/S, Denmark (Soluprick SQ®, batch Hu 073, containing freeze-dried dog dander and hair from different dog breeds. 1 mg/ml corresponding approximately 1:20 w/v, 1 HEP). The same batch of this allergen was also used for the provocation tests (as an aqueous extract), the Al-RAST and paper RAST determinations. A dog epithelium extract e2 (referred to as allergen e2) and a dog dandruff extract e5 (referred to as allergen e5) (Pharmacia Diagnostics AB, Sweden) were used in the Phadebas RAST.

Skin prick tests

To standardize the evaluation of the results, the method recommended by the Northern Society for Allergology (1) was followed. Each skin test reaction was compared with the negative control (the diluent provided by the manufacturer). A weal size equal to the reaction of the positive control (histamine hydrochloride 1 mg/ml) was recorded as +++ reaction. The skin reactions were recorded after 15–20 min.

Provocation tests

All patients were investigated by the provocation test. Most of the patients (89%) were tested by the bronchial provocation test (BPT). Those (a) with a low peak expiratory flow (PEF $\leq 60\%$ of the expected value) (17), (b) those reacting to the control diluent with a decrease of $\geq 15\%$ PEF, (c) those with positive auscultatory findings (rales, wheezing), and those (d) lacking sufficient co-operation were excluded from the BPT. Instead of BPT the conjunctival provocation test (CPT) was performed on these 24 patients. CPT was also carried out on patients with a doubtful BPT result and on those with a suggested dog dander allergy but a negative BPT result. BPT and CPT were performed as described elsewhere (18).

RAST analyses

A blood sample for RAST analyses was obtained from each subject before the skin testing. Sera were stored at -20°C until the determinations were performed.

Phadebas RAST analyses were done with e2 and e5 dog dander and hair allergen preparations using commercially available *Phadebas* RAST materials. An ξ_2 -specific ^{125}I labelled anti-IgE was used (7).

Paper RAST was performed with Hu 073 coupled to activated filter paper discs according to Ceska et al. (6). The coupling was performed in an allergen extract concentration of 2 mg/ml. Immunoabsorbed rabbit anti-human IgE (ξ -chain specific) was prepared as described elsewhere (15); labelling with ^{125}I was performed by means of the chloramin T method (9). The results were expressed as a percentage of the total activity.

Al-RAST was performed according to Weeke* (19).

Briefly it was carried out as follows:

200 μl Hu 073 (2 mg/ml) were absorbed to 50 μl Alhydrogel® (Superfos, Vedbæk, Denmark) containing 2% (w/v) $\text{Al}(\text{OH})_3$ (equivalent to 1.3% (w/v) Al_2O_3) and were dissolved in 2 ml coupling-buffer (TRIS, HCl 0.1 M, pH 7.5, with 1% (v/v) Tween 20 and 0.015 M NaN_3).

The suspension was shaken gently for 15 min at room temperature and centrifuged at 3000 rpm for 2 min. The supernatant was gently sucked away leaving about 0.2 ml on top of the precipitate. This washing procedure was repeated four times using 2.5 ml coupling-buffer. After washing, the precipitate was resolved in 5 ml coupling-buffer and was now ready for use. The quantity containing 100 μl of the well-mixed suspension was distributed in small plastic tubes and a 50 μl serum sample was applied and allowed to react for 16 h at room temperature.

A coupling-buffer (2.5 ml) was added, the tubes were centrifuged at 3000 rpm for 2 min, followed by suction leaving 0.3 ml free supernatant. This was repeated three times.

After the fourth washing procedure all tubes were turned upside down for a few seconds in order to decant the free supernatant.

Anti-IgE (50 μl) labelled with ^{125}I (approximately 120,000 cpm) was added to the tubes. After 16 h at room temperature 2.5 ml coupling-buffer was added. The mixture was centrifuged and washed as previously described. After the fourth washing procedure the last 0.3 ml free supernatant was left on the top of the precipitate.

Finally, the amount of specific labelled anti-IgE bound to the allergen at the Alhydrogel was counted (5 min).

As reference system was used serum from a patient highly allergic to *Phleum pratense* standardized as a 100 Sorbent Unit (SU) reference (20). The standard curve presented a straight line in a double log scale with counts as y values and dilutions of the reference serum as x values diluted with normal serum reference (sera from 1000 donors randomly chosen and pooled).

The results were expressed as a percentage of the total activity or in Sorbent Units (SU) and in allergy classes (class 0, 1, 2, 3 and 4 corresponding to < 2 SU, < 4 SU, < 20 SU, < 100 SU and ≥ 100 SU, respectively). The grading system corresponds to that of the *Phadebas* RAST reference system using a timothy system instead of a birch system.

Serum IgE determinations

Total IgE levels in all sera were determined by *Phadebas*® IgE PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden).

Allergy score

The results of the clinical history, the skin prick test and the provocation test were combined to obtain a score system for comparing the different RAST systems. The scores of each subject were counted by summing up the points from each test as follows:

* Patent No. 140.815, Denmark.

Score points	Clinical history	Skin prick test	Provocation test
0	negative	negative	negative
1	doubtful	+ or ++	doubtful
2	positive	+++	positive with conc. 1 mg/ml
3		++++	positive with conc. <mg/ml

For purposes of this study, the subjects with an allergy score of at least four points were arbitrarily considered as dog allergic (D+) subjects. Those with an allergy score of 0–3 points were considered as non-allergic to dog (D–). Accordingly, the sensitivity, specificity and overall test efficiency (8) were defined as follows:

$$\text{sensitivity} = \frac{\text{No. of D+ cases with RAST class 2-4}}{\text{No. of D+ cases}}$$

$$\text{specificity} = \frac{\text{No. of D- cases with RAST class 0-1}}{\text{No. of D- cases}}$$

$$\text{overall test efficiency} = \frac{\text{No. of D+}_{\text{RAST 2-4}} + \text{No. of D-}_{\text{RAST 0-1}}}{\text{No. of total assays}}$$

Statistical methods

The statistical significance of the differences between the two means was tested by using the Student's *t*-test after having applied the *F*-test.

The statistical significance of the differences between the observed group distributions was tested by using the χ^2 -test. In the fourfold tables Yates' correction for continuity was used. The correlation coefficients were calculated with the aid of Pearson's formula for the product-moment correlation coefficient.

RESULTS

Specific IgE to dog dander and hair was observed in most of the asthmatic children, when tested with Phadebas RAST e5 or Al-RAST (Fig. 1); Phadebas RAST e2 was less sensitive yielding a class 0 result in 51.5% of the children. By contrast, Al-RAST gave a class 0 result in only one case out of these 200 un-

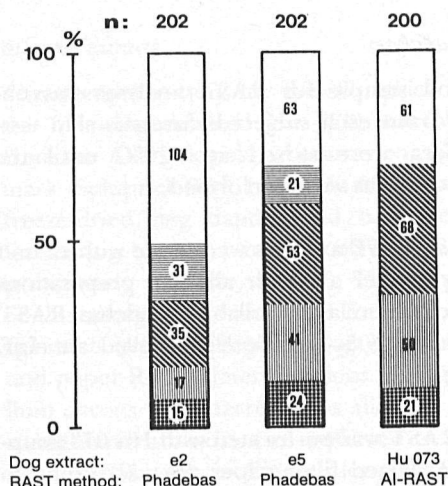
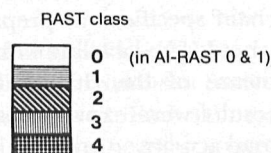


Fig. 1. Distribution of the results in various RAST determinations. Explanation of the symbols:



selected asthmatic children. Since different reference systems are used in Phadebas RAST and Al-RAST, the classes are not directly comparable, e.g. class 2 in Al-RAST is not exactly equal to class 2 in Phadebas RAST.

RAST compared with clinical history

Both RAST methods, Al-RAST and Phadebas RAST, gave results which were in good agreement ($P < 0.001$) with the clinical history of dog allergy (Fig. 2). However, Phadebas RAST with allergen extract e2 gave negative (class 0) results (30.4%) about three times more often in patients with a positive clinical history than Phadebas RAST e5 (10.1%). On the other hand, a class 1 result with e5 RAST was seldom combined with a positive clinical history. A class 4 result was found with none of the methods in subjects with a negative clinical history.

RAST compared with skin prick test (SPT)

A good agreement ($P < 0.001$) was generally observed between RAST and SPT (Fig. 3). A

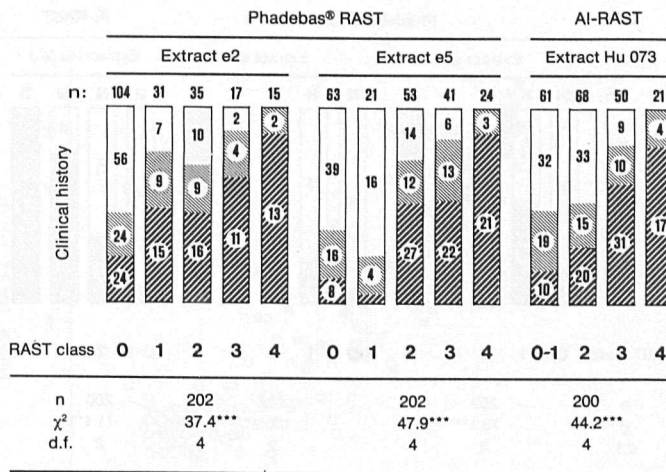


Fig. 2. The distribution of positive (▨), doubtful (▤) and negative (○) clinical history by RAST class.
*** $P < 0.001$.

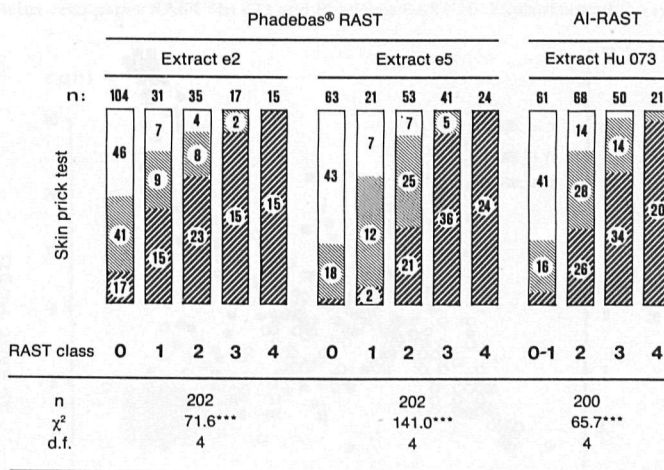


Fig. 3. The distribution of strongly positive (▨), slightly positive (▤) and negative (○) skin test results by RAST class. A skin prick test result $\geq +++$ is regarded as strongly positive and a result + or ++ as slightly positive.
*** $P < 0.001$.

positive SPT result was observed in 71.8% of the subjects; thus SPT seems to have about the same sensitivity as Phadebas RAST or AI-RAST. However, there were a few children with a strong ($\geq +++$) skin test reaction but a negative RAST result, while the reverse, i.e. subjects with a clearly positive (class 3–4) Phadebas RAST but a negative skin test, was not observed.

RAST compared with provocation tests

The results of the provocation tests correlated well with the RAST results (Fig. 4). 70.2% of the subjects with a positive provocation test reaction had a Phadebas RAST result class 3–4 with allergen e5, but only 34.5% with allergen e2. 67.1% of the subjects with a positive provocation test reaction had an AI-RAST result class

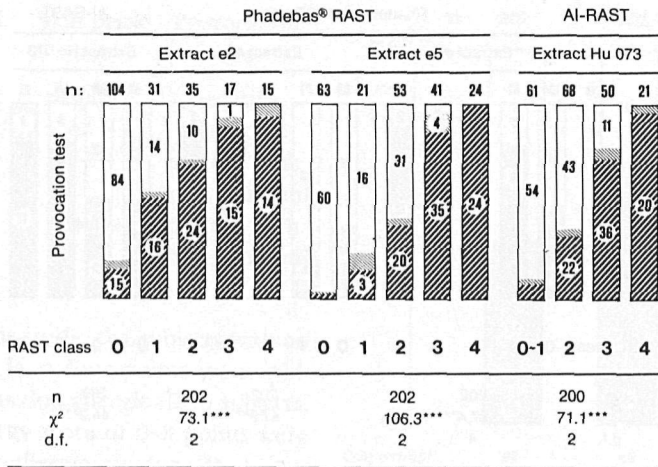


Fig. 4. The distribution positive (■), doubtful (●) and negative (○) provocation test results by RAST class.
*** $P < 0.001$.

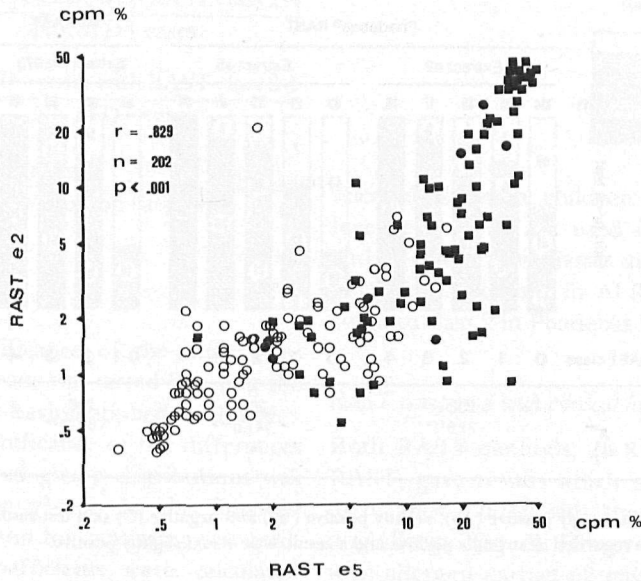


Fig. 5. Correlation between Phadebas RAST e2 and e5. Explanation of the symbols:

- = positive provocation test result.
- = negative provocation test result.
- = doubtful provocation test result.

3–4. Generally those with a negative RAST with any of the tests had also a negative provocation test. However, e2 RAST gave a class 0 result in 15/84 (18%) of the cases with a positive provocation test.

Comparison of RAST results

The correlation of the various RAST methods using different commercial dog dander extracts was generally very good. Allergen extracts e2

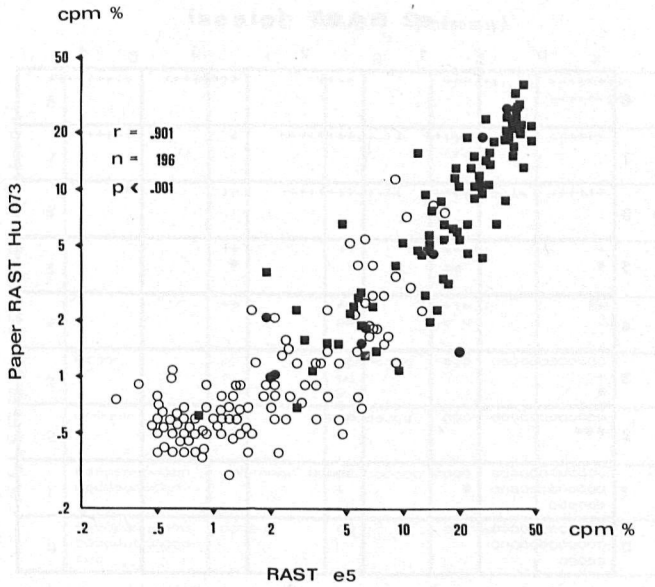


Fig. 6. Correlation between paper RAST Hu 073 and Phadebas RAST e5. Explanation of the symbols: see Fig. 5.

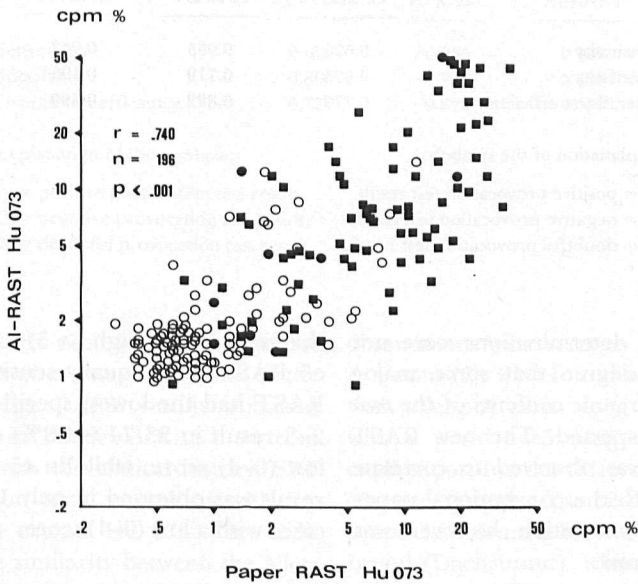


Fig. 7. Correlation between paper RAST and AI-RAST using the same allergen extract (Hu 073). Explanation of the symbols: see Fig. 5.

and e5 gave fairly consistent results in Phadebas RAST determinations (Fig. 5). There were, however, a number of subjects with rather low counts in e2 RAST but high counts in e5 RAST suggesting a possible defect in the allergenic contents of the e2 extract. (The differences be-

tween e2 and e5 RAST results could not be explained by the variations in the total IgE levels of the subjects).

The correlation between Phadebas with allergen extract e5 and paper RAST with extract Hu 073 was excellent (Fig. 6). Clearly differing

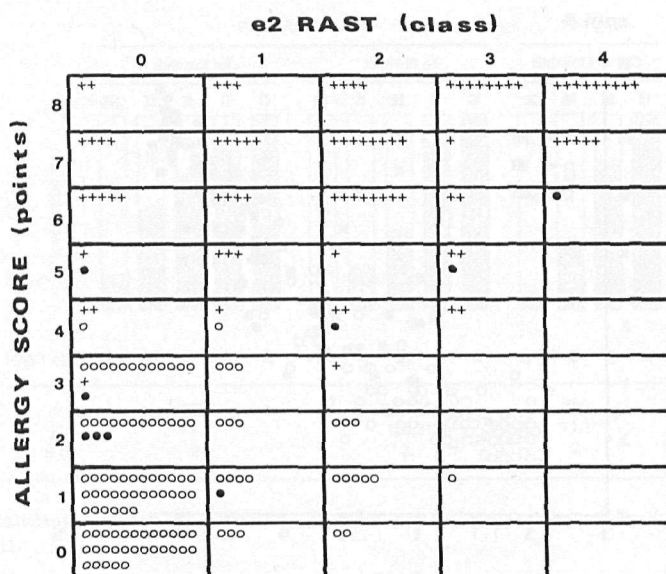


Fig. 8a. Various RAST determinations compared with allergy score.

	e2 RAST	e5 RAST	AI-RAST
Sensitivity	0.625	0.955	0.943
Specificity	0.895	0.719	0.496
Overall test efficiency	0.777	0.822	0.690

Explanation of the symbols:

- + = positive provocation test result.
- = negative provocation test result.
- = doubtful provocation test result.

counts between these determinations were not observed to such a degree that some major differences in the allergenic contents of the two extracts could be suspected. The new RAST method, AI-RAST, was observed to correlate fairly well (Fig. 7) with the conventional paper RAST method. The correlation, however, was poorer with lower counts.

RAST compared with allergy score

The allergy score combined the results of the clinical history, the skin prick test and the provocation test. Some differences were observed between the different RAST systems when compared with the allergy score (Fig. 8 a-c). e2 RAST had the best specificity, but poor sensitivity: it gave a class 0 result in 11/69 (16%) of

the cases with a high (> 5) score. AI-RAST and e5 RAST were equally sensitive. However, AI-RAST had the lowest specificity: it gave a class 2-3 result in 33/74 (44.6%) of the cases with a low (0-1) score, while in e5 RAST a class 2-3 result was observed in only 12/75 (16%) of the cases with a low (0-1) score.

DISCUSSION

The purpose of the present study was to compare three purified dog dander and hair extracts using the conventional paper RAST (Phadebas RAST) and a modification of RAST, AI-RAST. The *in vivo* tests, SPT and the provocation tests were performed using only one of the allergen extracts (Hu 073), because the

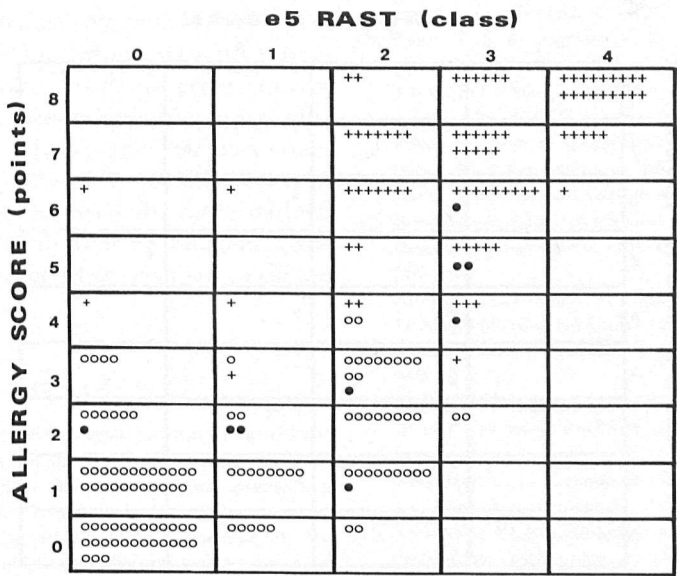


Fig. 8b. Various RAST determinations compared with allergy score.

	e2 RAST	e5 RAST	AI-RAST
Sensitivity	0.625	0.955	0.943
Specificity	0.895	0.719	0.496
Overall test efficiency	0.777	0.822	0.690

Explanation of the symbols:

+ = positive provocation test result.

○ = negative provocation test result.

● = doubtful provocation test result.

other allergen extracts used in RAST were not available for the *in vivo* tests at the time of the study. However, the RAST results obtained using allergen e5 in Phadebas RAST were in good agreement with the clinical history, SPT and the provocation tests, as well as with the RAST by allergen extract Hu 073. This indicates a remarkable similarity between the allergenic contents of the e5 and Hu 073 extracts.

Allergen e2 was observed to give false negative results more often than the other two preparations used, suggesting a possible defect in its allergenic contents. This is in agreement with studies of Merrett & Merrett (16), who observed that one of the two commercially available RAST reagents, dog dandruff, was successful for screening by the RAST technique, but dog epithelium was unsuitable. Their

observations were recently confirmed by Brandt & Yman (5), who found a positive RAST result with allergen e2 in only 13/38 (34%) of those with a positive e5 (dog dander) RAST. One explanation for the differences between e2 and e5 extracts is that the e2 dog epithelium disc is prepared from the skin scrapings of one single breed (Dachshund), while e5 dog dander discs are prepared from mixed dog dander. On the other hand, e5 may give less specific results since dog dander contains an allergen cross-reacting with cat epithelium (5, 16). However, our unpublished results in these same subjects indicate that there is no significant difference between e2 and e5 in this respect: e2 also often gave positive results in cases with a positive history of cat allergy but a negative history of dog allergy.

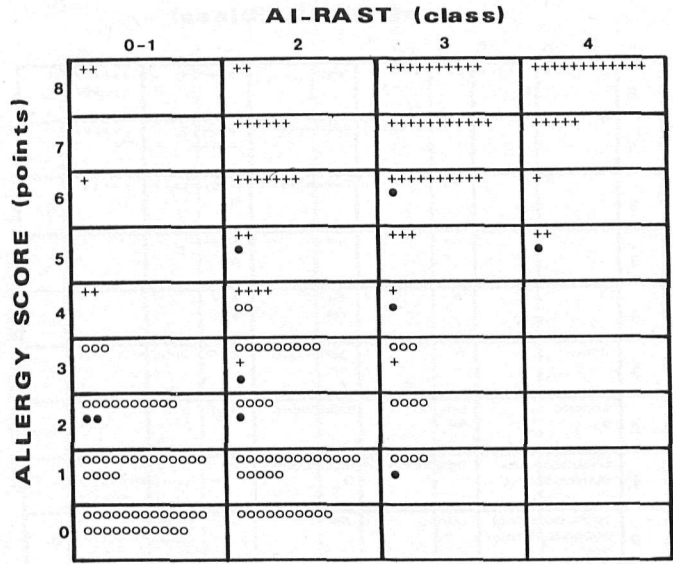


Fig. 8c. Various RAST determinations compared with allergy score.

	e2 RAST	e5 RAST	Al-RAST
Sensitivity	0.625	0.955	0.943
Specificity	0.895	0.719	0.496
Overall test efficiency	0.777	0.822	0.690

Explanation of the symbols:

+ = positive provocation test result.
○ = negative provocation test result.
● = doubtful provocation test result.

Most subjects with a RAST result class 1 or 2 gave a negative or doubtful result in the provocation test. It is possible that some of these cases with a negative provocation test would have reacted positively, if more concentrated test solutions had been used, or repeated provocation tests had been made, or another target organ had been selected for the test (18). Another explanation for the minor discrepancies between the provocation test results and RAST could be the presence of IgG antibodies, which have been observed in asthmatic subjects allergic to cat dander (11).

In Phadebas RAST, classes 1 and 2 were mostly found in subjects with a negative result in the provocation test; thus these classes can be regarded as clinically doubtful where dog allergy is concerned. Classes 3 and 4 were mostly

combined with a positive provocation test result and a positive clinical history, and thus can be regarded as clinically significant. Phadebas RAST e2 was less sensitive than e5 RAST and therefore most patients with e2 RAST class 1-4 gave a positive reaction in the provocation test. In Al-RAST, classes 0 and 1 can be considered as clinically negative, class 2 as doubtful and classes 3 and 4 as positive. Thus, with good allergen extracts a RAST result 3-4 can be regarded as clinically significant, but the clinical diagnosis should be based on several diagnostic criteria. A scoring system similar to our allergy score can be used to eliminate the possible limitations of a single test.

At present many of the commercial allergen extracts used in clinical work are not standardized and may give results not in full agree-

ment with the available commercial RAST products (2, 3, 14). Our results show that well-standardized extracts from different sources may be used interchangeably in the *in vivo* tests and in the RAST. However, even these standardized extracts showed minor differences in RAST, suggesting the need for more defined immunochemical analysis of the antigenic composition of the allergen extracts used in clinical work.

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