

Reproducibility of skin prick testing with allergen extracts from different manufacturers

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Allergen extracts for skin prick testing (SPT) are available from several manufacturers. Although these solutions are specified according to well-defined internal company standards, there is no generally accepted overall standardization. To assess the comparability of skin prick test solutions of various manufacturers, we compared extracts of nine different allergens from four companies by SPT in 29 children sensitive to one or more of the allergens, in a double blind fashion. RAST (Pharmacia) and EAST (Kallestad) were determined in simultaneous blood samples. Allergen extracts were also examined by rocket immunoelectrophoresis for their content of major allergens. Skin reactions, assessed by mean diameter of wheals, to identical allergen extracts varied significantly between the four vendors ($P < 0.05-0.001$). Correlation between RAST and EAST was good for all allergens except birch pollen and mugwort. Content of the major allergen in corresponding extracts varied significantly between the different companies ($< 1\%-2000\%$). These data underline the need for international reference extracts as intracompany standardization of test solutions alone is not enough to yield general reproducibility of skin prick test results.

Key words: allergen extracts; different manufacturers; reproducibility; skin prick test.

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Skin prick testing (SPT) is widely used for the diagnosis of IgE mediated hypersensitivity and for evaluation of the biological activity of allergen extracts (6).

However, results of SPT obtained in different centers vary considerably, depending not only on methodology, type of needle used and experience of the person carrying out the test, but also on the allergen extract used. Currently, most of the commercially available allergen extracts are specified according to well-defined internal company standards. But despite recent efforts to establish international standards, only a few generally accepted reference preparations are available so far (18).

The aim of this study was to assess the comparability of SPT solutions marketed by various companies.

PATIENTS AND METHODS

Standardised SPT (13) with a 1 mm SPT needle (Allergy Pricker, Dome/Hollister-Stier) was performed in 29 children (23 boys and 6 girls), 4-19 years old, who were known to be sensitive to one or more of the employed allergens. The diagnosis of allergy was based on history, previous SPT and RAST. None was treated with drugs which are recognised to interfere with SPT results (16).

Nine different inhalant allergens (*Dermaphagoides pteronyssinus*, cat-, dog-, guinea pig dander, birch pollen, mixed grasses, rye, mugwort and *cladosporium*) and histamine as a positive control, from four different companies (Abello, Spain; ALK, Denmark; Allergopharma, F.R.G., Bencard, U.K.) were simultaneously applied to the subjects' backs as

single determinations in a double-blind manner. Reproducibility of double determinations was found to be high in a previous study (7). The SPT solutions were reconstituted in glycerol diluent and were labelled by the manufacturers as follows: BU or w/v (Abello), HEP (histamine equivalent in prick test) or w/v (ALK), PNU (Allergopharma) and w/v (Bencard). The concentrations of the allergen extracts used appear from Table 1. Positive references were histamine dihydrochloride at a concentration of 1 mg/ml (Abello, Allergopharma and Bencard) and 2 mg/ml (ALK) in glycerol diluent. The negative control solution was 50% glycerol.

Skin reactions were read 20 min after allergen application and expressed as the mean of two perpendicular diameters of wheals. The outline of the wheal was drawn on the skin with a ball-point pen, then traced on to a piece of transparent tape, which was attached to paper. The measurements were performed by one blinded person at the completion of the study.

RAST (Pharmacia, Sweden) and Enzyme Allergosorbent Test (EAST, Kallestad, USA) were determined simultaneously on blood samples drawn at the time of testing according to the companies' specifications.

The following extracts of three companies (Abello, ALK, Bencard) were examined for their major allergen content by rocket immunoelectrophoresis at the Protein Laboratory, University of Copenhagen, Denmark: antigen 42 of *Dermatophagoides pteronyssinus*, antigen 4 of cat dander, antigen 13 of dog dander, antigen 23 of birch pollen and antigen 20 of mugwort (1).

Statistics

Analysis of variance was performed for comparison of SPT reactivity. RAST and EAST results were compared by coefficient of correlation. Kendall coefficient of correlation was used for comparison of SPT reactions and content of major allergen. Wilcoxon signed rank test was used to test for significance of differences in SPT results between standardized allergen extracts. $P < 0.05$ was considered the level of significance.

RESULTS

Skin reactions to histamine varied significantly between the four different solutions with mean diameters from 2.3–4.1 mm, $P < 0.001$ (Fig. 1).

Table 1
The concentrations of allergen extracts used

Extract	Company			
	I	II	III (PNU/ml)	IV (% w/v)
<i>D. pt.</i>	100 BU/ml	100,000 SQE/ml (= 3 HEP)	1000 PNU/ml	1.2% w/v
Cat dander	1% w/v	100,000 SQE/ml (= 3 HEP)	5000 PNU/ml	150% w/v
Dog dander	1% w/v	100,000 SQE/ml (= 3 HEP)	5000 PNU/ml	150% w/v
Guinea pig dander	1% w/v	1:30 w/v	5000 PNU/ml	6% w/v
Cladosporium	1% w/v	1:20 w/v	5000 PNU/ml	10% w/v
Mixed grasses	100 BU/ml	100,000 SQE/ml (= 3 HEP)	10000 PNU/ml	2.5% w/v
Rye	1% w/v	100,000 SQE/ml (= 3 HEP)	5000 PNU/ml	2.5% w/v
Birch pollen	2.5% w/v	100,000 SQE/ml (= 3 HEP)	5000 PNU/ml	5% w/v
Mugwort	100 BU/ml	100,000 SQE/ml (= 3 HEP)	5000 PNU/ml	2.5% w/v

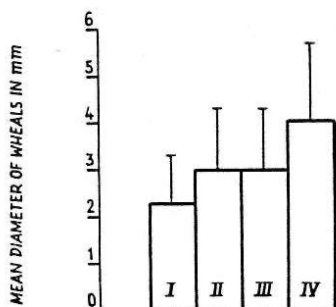


Fig. 1. Skin reactions to four different histamine solutions, expressed as mean wheal diameters $\pm$ SD, varying significantly from 2.3 mm to 4.1 mm ($P < 0.001$).

I Abello (0.1%), II ALK (0.2%), III Allergopharma (0.1%), IV Bencard (0.1%).

The negative control solution did not induce wheals in any patients.

Fig. 2 shows the differences of mean wheal diameters of the nine allergen extracts from the four manufacturers. Consistent skin reactions were found for house dust mite, birch pollen and cladosporium. However, for the other six allergen extracts, wheal sizes varied significantly ($P < 0.05$ – 0.001), especially for the animal dander extracts. Analysing standardized extracts alone, indicated by a star, good inter-company comparability was found for house dust mite and mugwort; but skin reactions to mixed grasses varied significantly ($P < 0.001$).

Table 2 shows the correlation between RAST and EAST results. With the exception of birch pollen ($r = 0.51$) and mugwort ($r = 0.49$) there was a good agreement between the radioimmunological and the enzymatic methods ($r = 0.78$ – 0.97).

In Table 3 the numbers of both positive and negative SPT, RAST and EAST results are listed. RAST and EAST classes 2–4 were defined as positive, classes 0–1 as negative. EAST determinations were performed in only 22–24 patients. A skin reaction $\geq 75\%$ of the diameter produced by histamine was defined as positive, one $\leq 50\%$ of the histamine reaction as negative. Agreement was found to be good only for house dust mite and birch pollen, but lacked especially for cat-, dog dander and mixed grasses.

The results of the rocket immunoelectrophoresis are given in Table 4. The concentrations of the extracts of Abello and Bencard are related to ALK solutions, which were used as standard, with their labelled potency of 3 HEP as 100%. The content of one major allergen varied between 100%–2000% for Bencard's test solutions and between $< 1\%$ and 1000% for Abello's extracts.

Relating mean wheal diameters of the individual allergen extracts to the content of one major allergen, a significant correlation was found for cat dander ($r = 0.43$, $P < 0.001$) and dog dander ($r = 0.26$, $P < 0.005$). No correlation was found for house dust mite, birch pollen and mugwort.

DISCUSSION

The present study compared the skin reactivity to nine of the most common allergens in central Europe, using allergen extracts from four different companies, some of which were standardized by the manufacturer and some were not.

Although three histamine solutions had the same concentrations of 0.1%, varying skin prick reactions were obtained. We do not think that the observed differences can be solely explained by human error, since SPT was always performed by the same experienced nurses and all measurements were done by one person at the study's end. One explanation may be the low concentration used, which produced small wheals (14). Recently, higher reproducibility has been reported by using histamine 10 mg/ml instead of 1 mg/ml (19).

Table 2
Comparison of RAST/EAST

Allergen	RAST/EAST
	Coefficient of correlation (r)
<i>D. pt.</i>	0.83
Cat dander	0.87
Dog dander	0.93
Guinea pig dander	0.86
Cladosporium	0.78
Mixed grasses	0.97
Rye	0.89
Birch pollen	0.51
Mugwort	0.49

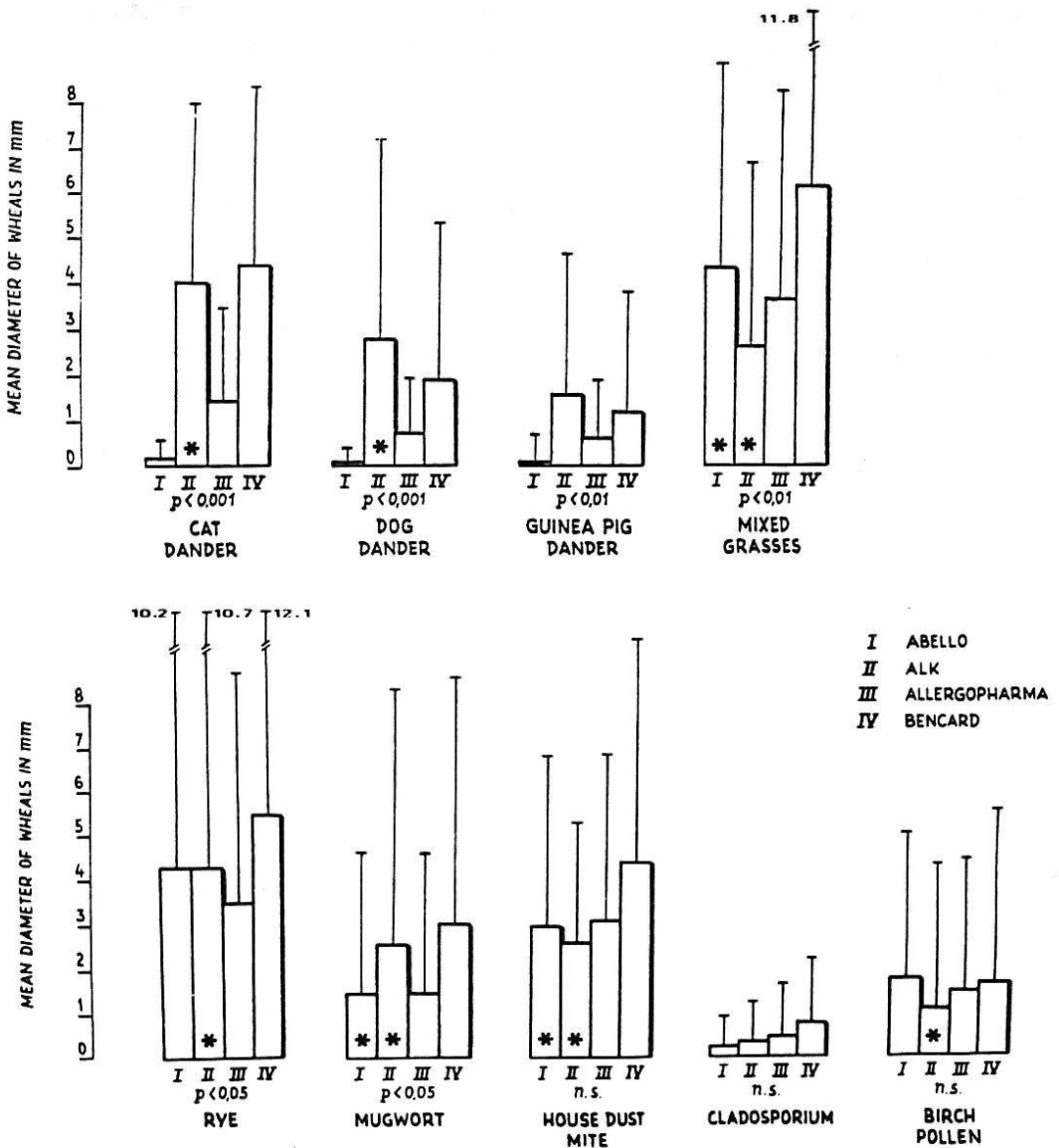


Fig. 2. Comparison of mean wheal diameters $\pm$ SD of nine allergen extracts from four companies.

Significant differences were found for cat- and dog dander ($P < 0.001$), guinea pig dander, mixed grasses ($P < 0.01$), rye and mugwort ($P < 0.05$). Skin reactions to house dust mite, cladosporium and birch pollen did not differ significantly. Standardised allergen extracts are indicated by a star. Skin reactions to mixed grasses between the standardized extracts of Abello and ALK varied significantly ($P < 0.001$); no differences were found for house dust mite and mugwort.

Mean wheals sizes also varied significantly for six of the nine allergen solutions (Fig. 2). Additionally, the statistically insignificant differences for cladosporium and - partly - birch pollen extracts may very well conceal considerable real

differences between the products since only few patients demonstrated a measurable reaction. In those extracts with well-defined major allergens (3, 4, 10, 11, 12), we found considerable inter-company variations in major allergen content

when assessed by RIE (Table 4). Thus skin test results could not be expected to be in accordance. Abello's extracts of cat- and dog dander, with very low content of Ag 4 and Ag 13 respectively, elicited significantly smaller wheals than the other more concentrated solutions. Relating the mean wheal diameter to the content of major allergen there was a significant correlation for the extracts of these two allergens. However, increasing the major allergen concentration above a certain level does not necessarily correlate with an increase in wheal size, as the histamine reserve of mast cells may be exhausted above a certain concentration (8). This could explain why the markedly higher major allergen content of birch pollen and house dust mite solutions from Abello and Bencard did not produce significantly larger wheals. This is also emphasized by no significant

correlation of SPT wheal reaction and content of major allergen for these allergen extracts.

Specifying allergen extracts to internal company standards apparently does not always result in good intercompany comparability as we found significant differences between the standardized extracts of mixed grasses from Abello and ALK.

We also compared SPT results to corresponding serum IgE antibody titers as determined by RAST and/or EAST by listing the numbers of positive and negative results from each test. Intercompany agreement should be expected to a high degree for corresponding allergen extracts of similar SPT potency or major allergen content. In accordance with our SPT results, good intercompany agreement was found for house dust mite and birch pollen but lacked especially for cat-, dog dander and mixed grasses. These dis-

Table 3

Numbers of positive and negative SPT results obtained with extracts from four different manufacturers (I-IV) and of EAST and RAST results. For definition of positive and negative reactions see Results

	SPT								EAST		RAST	
	I		II		III		IV		+	-	+	-
	+	-	+	-	+	-	+	-				
<i>D. pt.</i>	13	16	11	18	13	16	12	17	13	11	16	13
Cat	0	29	15	14	7	22	14	15	15	9	15	14
Dog	0	29	7	22	2	27	4	25	7	16	9	20
Guinea-pig	1	28	4	25	2	27	3	26	2	20	3	26
Cladosporium	2	27	2	27	2	27	1	28	3	20	4	25
Birch pollen	7	22	5	24	7	22	5	24	10	12	5	24
Mixed grasses	18	11	11	19	12	17	16	13	15	8	18	11
Rye	15	14	10	19	11	18	14	15	15	8	17	12
Mugwort	5	24	4	25	4	25	6	23	6	16	10	19

Table 4

Comparison of five allergen extracts from 3 manufacturers with regard to major allergen content as determined by RIE

Allergen	ALK (II)	Bencard (IV)	Abello (I)
<i>D. pt.</i>	3 HEP = 100%	2000%	500%
(Ag 42)		declared 1.2% w/v	declared 100 BU/ml
Cat dander	3 HEP = 100%	200%	< 1%
(Ag 4)		declared 150% w/v	declared 1% w/v
Dog dander	3 HEP = 100%	200%	< 1%
(Ag 13)		declared 150% w/v	declared 1% w/v
Birch pollen	3 HEP = 100%	1000%	1000%
(Ag 23)		declared 5% w/v	declared 2.5% w/v
Mugwort	3 HEP = 100%	100%	10%
(Ag 20)		declared 2.5% w/v	declared 100 BU/ml
varying composition!			

crepancies cannot be explained by differences in RAST and EAST results, as correlation between these two methods was generally good (Table 2). Thus, *in vivo/in vitro* discrepancies probably reflect the highly varying major allergen content of the SPT extracts.

SPT is the most widely used method for the diagnosis of immediate type hypersensitivity. Provided the test is performed by the same person high consistency of SPT results can be obtained (19). However, a crucial point in assessing the value of the skin test as a diagnostic tool is the reproducibility between different laboratories, depending not only upon standardized SPT methodology and the use of standardized needles (2, 13, 17) but greatly upon consistent allergen content of extracts from different lots of one manufacturer and between different manufacturers. To achieve this goal, the use of equipotent standardized allergen extracts is mandatory.

We conclude from our results that biological and/or immunochemical specifications of allergen extracts to internal company standards are not sufficient to yield general reproducibility of SPT results. Intercompany standardization of allergen extracts by use of international reference allergen extracts and international reference serum pool in manufacturing of standardized extracts seems most likely to overcome these discrepancies (5, 9, 20).

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