

Allergy screening including a comparison of prick test results with allergen-coated lancets (Phazet®) and liquid extracts

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During an allergy screening of families with a history of allergy in one or more subjects, skin prick tests (SPT) were performed in duplicate in 314 adults. The tests were performed with a new type of lancet with a 0.9 mm long point, loaded either with allergen (Phazet®) or used together with standardized extracts (10,000 and 100,000 BU/ml). Wheals obtained with both methods were compared, and related to total IgE concentrations and history. A wheal area of 3 and 7 mm² was used as cut-off limit. Results with Phazet were close to those with Pharmalgen 100,000 BU/ml. With both methods precision was good for allergens, but less for Phazet histamine than for the corresponding wet test. SPT results obtained with Phazet and 100,000 BU/ml correlated better with history than the results with 10,000 BU/ml. Using a wheal size of 7 mm² as cut-off limit the efficiency for five tested allergens was 91 %. "False positive" tests, possibly indicating an unobserved or latent type of allergy, were found more often with higher serum IgE concentrations. The safety of SPT was confirmed and Phazet was well accepted by nurses and tested subjects.

Key words: adults; allergy; allergy pricker; IgE; skin prick tests.

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CLINICAL ASPECTS

As with any screening test, SPT should combine high sensitivity with the highest possible specificity. The specificity of Phazet and Pharmalgen 100,000 BU/ml was high with wheal areas > 7 mm² (diameter 3 mm) as the limit for positivity. Phazet and Pharmalgen 100,000 BU/ml showed equal sensitivity and precision. Phazet further simplifies the SPT procedure.

Once recommended (12), skin prick tests (SPT) replaced the intradermal test especially in pediatric allergy. To obtain positivity with SPT more potent allergen extracts are required than with intradermal tests. No deaths have been reported as a result of SPT, whereas there is an obvious risk of death when using intradermal tests (10). SPT results with a standard needle with a 1 mm point (11) are found reproducible (3, 11). If SPT is performed with this needle together with freeze dried standardized extracts, it is possible to

reliably compare results within the same subject from time to time and between samples of patients, and to investigate sensitization within and between populations.

Recently, a new skin prick test lancet, Phazet®, loaded with allergen has been introduced, and data on its calibration against a biologically standardized reference preparation, 100,000 biol. units/ml (BU/ml) has been presented (2). In this study we used total IgE and SPT with Phazet and allergen extracts to classify parents of allergy risk infants as atopic or non-atopic

(4). The aims of the present study were to compare wheal sizes and reproducibility with Phazet in tests using the corresponding extracts at 10,000 and 100,000 BU/ml. Furthermore, we wanted to find the most relevant concentration and wheal size to be used in SPT as evaluated in relation to clinical history and total IgE concentration.

MATERIAL AND METHODS

Investigated subjects

All parents of 212 allergy risk infants (i.e. with an immediate family history of atopic disease) enrolled in a study concerning diet during late pregnancy were addressed and informed of the purpose of the study (4). Three hundred and fourteen subjects volunteered to participate and gave their informed consent: 171 women and 143 men aged 18 to 42 years.

A careful history was taken regarding past and present atopic disease, and suspected causative allergens. For birch and timothy we accepted seasonal symptoms as a positive anamnesis, for dog, symptoms on contact, for mite, seasonal symptoms in the autumn or when making beds, and for *Cladosporium*, symptoms from July to snow-fall and in humid indoor environment. The "total diagnosis" was defined as the accumulated history of atopic disease.

No antihistamines, steroids or bronchodilators were allowed during the 48 h prior to skin testing.

Allergen extracts

Freeze-dried and standardized Pharmalgen® extracts (Pharmacia AB, Uppsala, Sweden) of timothy and birch pollen, dog, *Dermatophagoides pteronyssinus* and *Cladosporium* 10,000 and 100,000 BU/ml were used throughout the study. The freeze-dried material was reconstituted at least once a week (for *Cladosporium* every 3rd day) with Albumin diluent®. Histamine HCl 1 and 10 mg/ml (5.43 and 54.3 mmol/l, respectively) served as positive control

and the albumin diluent as negative control solutions.

Phazet

Phazet is a skin pricker with a 0.9 mm (± 0.15 mm) tip, covered with Pharmalgen®, allergens and histamine (Pharmacia AB, Uppsala, Sweden). The Phazet lancets were delivered in separate envelopes. The Phazet had previously been calibrated to equal the corresponding extract, 100,000 BU/ml (2). Unprepared Phazet prickers were used as negative controls. All Phazet prickers and allergen extracts were kept at +4 to +8°C when not used for tests.

Skin prick tests

All tests were performed in duplicate by three experienced and specially trained nurses. Six prick tests for each of the five allergens, histamine and negative control were applied to the forearms of each subject in a reversed pattern.

Tests with extracts (wet tests) as well as tests with Phazet were performed as previously described (3), i.e. the pricker was applied to the skin at 90° to the skin surface and pressed down until stopped by the "shoulders". After 15 min (for histamine: 12 min) the wheals were encircled with a filter tip pen. The contours were transferred to a protocol by means of transparent tape. Wheal areas were estimated with a digitizer. Mean wheal areas of 3 mm² (diameter approx. 2 mm), used by some investigators, and 7 mm² (diameter approx. 3 mm), recommended by most authors (3), were both used as cut-off for positive tests in the calculations.

IgE-analysis

Venous blood was drawn before the tests. Serum was separated after 1 h and kept frozen at -20°C until analysed using Phadebas IgE PRIST®.

Statistical analyses

The chi-square test was used to estimate the correlation of clinical history and test results.

The Mann-Whitney test was used to study differences in distribution of total IgE between samples of patients. Sensitivity and specificity were defined according to Galen & Gambino (5). The clinical history was regarded as true indicator of disease as no provocation tests were performed.

The protocol was approved by the Human Research Ethics Committee. Informed consent was obtained from all participants.

RESULTS

Of 314 tested subjects, 194 (61.8%) gave histories of past or present atopic disease and 120 were regarded non-atopic by their histories. The most common current disease was allergic rhinitis (41.7%) followed by bronchial asthma (10.8%) and atopic dermatitis (9.6%). Healed atopic dermatitis was reported by a further 5.4% of the group. All diseases were overrepresented among females (e.g. allergic rhinitis, females 49.1% and males 32.9%).

At SPT, a wheal size of 3 mm² or more was found in 54.1% of the tested subjects, most often with birch and timothy pollen (Table 1).

Among the 120 subjects with a negative history the occurrence of a mean wheal area of 3 mm² or more for at least one allergen, was 15.0% with 10,000 BU/ml and 19.2% with 100,000 BU/ml or Phazet. With a positive history there was a positive skin test in 72.2 and 78.8% respectively. The group showing positive tests were mostly subjects with allergic rhinitis (82.5 and 87.2% respectively). Among those with isolated atopic dermatitis the corresponding incidence was 31.6% and 36.8%.

Table 1

Frequency of positive skin prick tests (%) with the five allergens (largest wheal with any of the tests)

	> 3 mm ²	> 7 mm ²
Birch	36.6	32.5
Timothy	35.0	30.9
Dog	27.7	18.5
Derm. pter.	16.9	8.9
Cladosporium	4.8	3.2

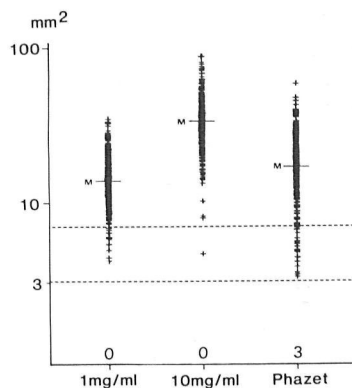


Fig. 1. Mean wheal areas (mm²) of duplicate tests in each patient with histamine HCl 1 mg/ml, 10 mg/ml and Phazet histamine. The horizontal bars indicate the respective medians. Figures in baseline indicate No. of negative SPT, i.e. < 3 mm² (2 mm in diameter).

The incidence of histamine wheals of more than 7 mm² varied between 89.2% (Phazet) and 99.4% (10 mg/ml) of the tested subjects (Fig. 1). In three subjects, the mean of two prick tests with Phazet histamine did not reach 3 mm². When comparing all tests performed with allergens, the concordance between Phazet and 10,000 BU/ml varied between the allergens from 81.2% to 96.5% (mean 89.4%) while it was somewhat higher when comparing Phazet with 100,000 BU/ml: 87.6% to 97.8% (mean 92.9%). The coefficient of correlation (r) for results obtained with Phazet and wet technique using timothy pollen allergen preparation was 0.81 to 0.88 and with dog allergen 0.64 to 0.74, using 10,000 and 100,000 BU/ml respectively (Fig. 2). When a wheal area > 3 mm² was regarded as the limit for positivity the frequency of discrepant findings between Phazet and the two wet methods was higher. However, the frequency varied strongly between allergens and discrepancies were most common for dog.

Relatively small differences were found in the correlation of test methods used to clinical history, with the exception of dog (Fig. 3) where Phazet appeared to give more "false positive" results.

The sensitivity and specificity of the different tests and of cut-off at 3 and 7 mm², was calcu-

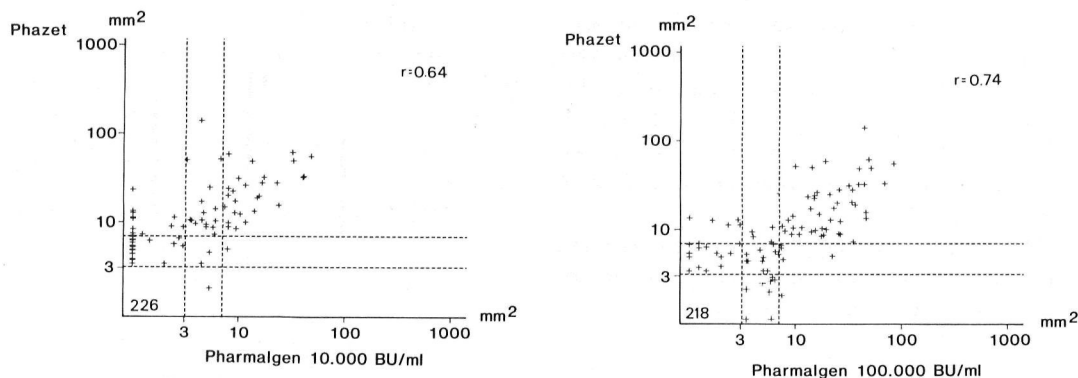


Fig. 2. Mean wheal areas (mm^2) of duplicate tests in each patient with dog allergen extract 10,000 BU/ml and Phazet dog (left) and with dog allergen extract 100,000 BU/ml and Phazet dog (right). Number of negative SPT are indicated in the left lower part of the figure.

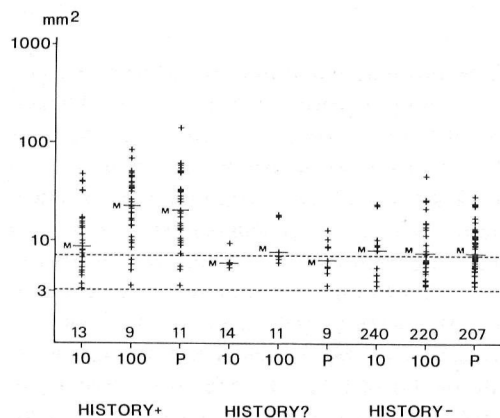


Fig. 3. Mean wheal areas of duplicate tests with dog allergen extract 10,000 BU/ml and 100,000 BU/ml and Phazet in relation to clinical history. The horizontal bars (M) indicate the median wheal area for each group.

lated for birch, timothy and dog versus the specific anamnesis (Table 2). Highest sensitivity was 84.3% and specificity 97.2%. The efficiency, calculated for all five allergens, was slightly higher for the cut-off limit 7 mm^2 (Phazet 91.6%, Pharmalgen 100,000 BU/ml 90.9%) than for 3 mm^2 (Phazet 88.1%, Pharmalgen 100,000 BU/ml 90.9%). For Pharmalgen 10,000 BU/ml the sensitivity was lower and the efficiency was 91.1% using 3 and 7 mm^2 as cut-off limits.

To explain "false positive" results a comparison was made between test results and the "total diagnosis" as well as with total IgE concentrations. For example, for Phazet birch pollen, a wheal $> 7 \text{ mm}^2$ was found in 102 of the subjects, 73 of whom had allergic rhinitis and a positive history for birch allergy. Only four of the positive tests were found in healthy subjects and 22 in subjects with allergic rhinitis but no obvious history of birch sensitivity. The incidence of positive birch pollen tests increased significantly with rising IgE concentrations regardless of the history (Fig. 4). Increasing incidence of positive tests with increasing IgE concentrations was also found when there was no history of allergy to timothy and dog. With IgE $> 120 \text{ kU/l}$ a "false positive" dog test was found in 51% of the subjects compared to 9.7% with IgE $< 50 \text{ kU/l}$.

Serum IgE concentrations were highest in subjects with bronchial asthma and immediate type gastrointestinal allergy (Table 3). In healthy subjects there was a decrease in IgE concentrations with age.

Coefficients of variation (CV) for extracts and Phazet showed small differences concerning allergen extracts (Fig. 5) with 100,000 BU/ml (0.52) showing the lowest CV. The CV for histamine was generally lower than that for allergens. The Phazet histamine gave a higher CV than the wet method with histamine HCl 1

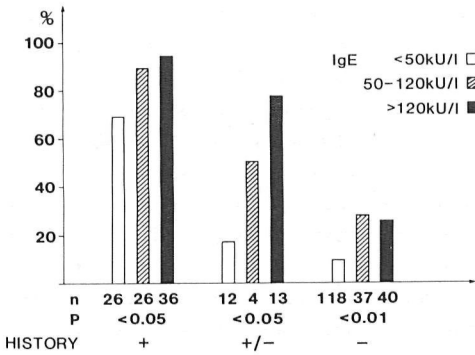


Fig. 4. Percent positive skin tests with either method against birch pollen in relation to serum IgE concentrations: < 50 kU/l (open bars) 50-120 kU/l (hatched bars) and > 120 kU/l (filled bars).

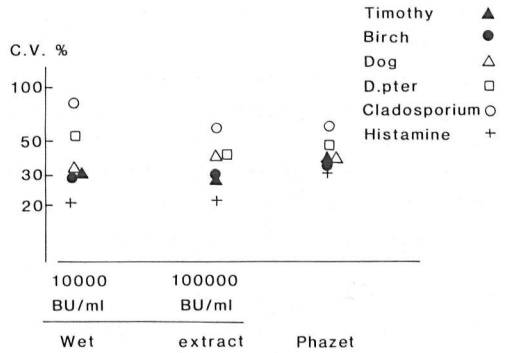


Fig. 5. The coefficient of variation of duplicate tests with the different test methods; birch (filled circles), dog (filled triangles), *D. pteronyssinus* (open circles) and histamine (crosses).

Table 2

The "sensitivity" and "specificity" at two cut-off limits for positive tests. A positive history was regarded as disease

	Birch		Timothy		Dog	
	3 mm ²	7 mm ²	3 mm ²	7 mm ²	3 mm ²	7 mm ²
10,000 BU/ml						
Sensitivity	80.9	76.4	77.4	57.1	69.8	46.6
Specificity	90.3	94.4	90.4	94.7	94.9	97.2
100,000 BU/ml						
Sensitivity	84.3	78.7	83.3	81.0	79.1	69.8
Specificity	85.7	89.8	86.6	90.0	87.0	93.3
Phazet						
Sensitivity	84.3	82.0	83.3	78.8	74.4	67.4
Specificity	88.8	91.3	87.1	90.9	81.8	90.1

Table 3

Serum IgE concentrations (geometric means) in kU/l and current diagnosis. Statistical comparisons were made between results in men/women and between age groups

	n	Total	Men	Women	Age (years)	
					20-29	30-40
Allergic rhinitis	132	78	59*	92	87	69
Bronchial asthma	34	166	224	134	154	192
Atopic dermatitis	30	110	181	91	66**	299
Gastrointestinal allergy	5	181	166	187	242	119
Healthy total	117	20	20	22	26	17
Healthy smokers	19	32	27	60	24	40
Healthy, non-smokers ¹	98	18	18	20	27*	15

¹95% confidence limits 14-24; ± 2 SD 2-217

*P < 0.05

**P < 0.01

and 10 mg respectively (0.41 as compared to 0.27 and 0.26).

Side effects in the form of slight general symptoms were reported by two of the tested subjects: a slight throat itch within 20 min but no other symptoms was treated with oral antihistamine, and in one of them a few puffs of epinephrin were added. Three subjects reported a dual skin reaction. The late reactions were treated with topical steroids.

The testing was well accepted by the subjects. Phazet was regarded easy to handle by the three testers and gave only minor discomfort to the tested subjects.

DISCUSSION

Our SPT results confirmed the results of the calibration of Phazet to corresponding extracts, i.e. the activity of the Phazet pricker is similar to that of Pharmalgen 100,000 BU/ml (2). The precision of Phazet was equal to that with the wet allergen extracts, and Phazet appears to be a useful innovation for allergy diagnosis. However, Phazet histamine showed a lower reproducibility than the wet tests and even a few negative results were found. Also among experienced nurses who perform many skin prick tests every week occasional tests may become false negative due to differences in application. We had expected the risk of false negative test results to be reduced by using preloaded prickers, as one step in the testing procedure would be omitted. Our three false negative tests out of 314 are indeed few, and perhaps the incidence will be even lower when Phazet has been used for a longer period. However, it may be better to test with Phazet histamine in duplicate to reduce the risk of false negative results, as it is important to include a positive reference in SPT in order to document the reactivity of the tested subject.

The coefficients of variation using histamine and allergen were similar to those obtained with the Allergy pricker (11) and in accordance with previous experience (7, 13). The higher variability found using Phazet histamine calls for extended investigations. Wheal sizes with Phazet histamine seem sufficient for checking

patient reactivity, but for standardization purposes a 10 mg/ml solution should be used as previously proposed (3, 15).

In this selected population with overrepresentation of atopic disease the incidence of positive skin tests was, of course, high. However, among the non-atopic subjects, the incidence of positive SPT was low even with the highest concentration of extract. We obtained a high "specificity" with most of the allergens tested. However, positive results were obtained with dog 100,000 BU/ml and Phazet dog in many subjects with no history of dog allergy. Therefore we will investigate these patients further, including RAST and provocation tests. As no provocation was performed, the true sensitivity of tests has not been elucidated.

A concentration of Pharmalgen extracts equal to or higher than 50,000 BU/ml was recently proposed for SPT (14) on the basis of a study in pollen-allergic subjects where SPT results were compared with conjunctival and nasal provocation tests. From our study it appears ideal to combine the use of 100,000 BU/ml or Phazet with a cut-off between positive and negative tests at 7 mm² (diameter of wheal = 3 mm) giving an efficiency (correctly diagnosed compared with history) of 91 % compared to 88 % if all wheals > 3 mm² were regarded positive.

Among "false positive" tests, most occurred in subjects with allergic rhinitis or other atopic disease and may well represent current allergy. In others it may represent a latent allergy (8). Interestingly, a high IgE concentration was found to increase the risk for a positive SPT, also when there was no specific history of allergy. As both may precede a subsequently developing allergy (3, 9) follow-up studies should be performed before it is possible to classify a test as false positive. To us, a high sensitivity is preferable for a screening test like SPT as long as the result is regarded as a sign of sensitization, not clinical allergy.

The levels of IgE-concentrations were in accordance with previous studies, but the IgE-concentrations found in healthy non-smokers are considerably lower than those reported by, e.g. Wittig et al. (16), thus stressing the necessity to consider smoking habits (1, 6, 17).

The low number of complaints and side effects in spite of massive SPT with potent extracts in even highly sensitive subjects is a further proof of the safety of SPT. All the nurses accepted the Phazet and even preferred it to wet tests. As Phazet is simple to handle and gives the same information as wet tests it seems especially suitable for use in small children and in field studies. The Phazet may reduce the exposure of nurses and others involved in allergy investigations as less allergen is spread in the surroundings.

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