

Comparison between modified skin prick test with standardized allergen extracts and Phazet

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Skin prick tests (SPTs) represent one of the most common techniques of skin testing and are of great value, especially when standardized extracts are used. Phazet is a puncture test involving needles coated with standardized allergens. Phazet and SPTs have been compared in 130 subjects tested with six standardized allergen extracts: orchard-grass, olive, and mugwort pollens, cat dander, Dermatophagoides pteronyssinus, and Alternaria. Patients have been carefully selected on the basis of a suggestive clinical history, SPTs, and RAST. For all allergenic species, at least 20 nonsensitive individuals have been tested. The sensitivity (true positive in percent of patients with disease) of Phazet was 99.4%, and its specificity (true negative in percent of patients without disease) was 99.2%. It is concluded that Phazet and SPTs with standardized extracts are equally effective in the diagnosis of immediate-type allergy and that standardized extracts have improved the diagnosis of allergy, especially for previously poorly characterized extracts, such as Alternaria. (J ALLERGY CLIN IMMUNOL 1988;82:878-81.)

SPTs are among the skin test methods from which to choose,¹ and, at least in Europe, they tend to be preferred to intradermal tests.^{2,3} The modified SPT introduced by Pepys and Bernstein³ is the current reference method, although the variability of this test has been demonstrated to be greater than that of the intradermal test.^{4,5} Many puncture tests have been proposed by different investigators during the past 10 years to decrease the variability of SPT. In previous studies it was found that Phazet (Pharmalgen, Pharmacia Diagnostics AB, Uppsala, Sweden) was a highly reproducible puncture test⁶⁻⁹ with a coefficient of variation with histamine or codeine phosphate <15%.⁶

Orchard-grass (*Dactylis glomerata*), olive (*Olea europaea*), and mugwort (*Artemisia vulgaris*) pollens, *Dermatophagoides pteronyssinus*, cat, and *Alternaria* are among the most common allergens of the Mediterranean area. These extracts are now standardized, and their shelf life is known; therefore, they can be used for comparisons of diagnostic methods.

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Abbreviation used

SPT: Skin prick test

Coated Phazet was compared with the modified SPT in patients allergic to these six allergen species to test the sensitivity and specificity of the newly described device.

MATERIAL AND METHODS

Subjects

One hundred 30 subjects ranging in age from 6 to 61 years (25.1 ± 12.4 years) were tested. All subjects had a full questionnaire of the personal and familial history of atopic disease, that is, asthma and/or rhinitis and/or conjunctivitis, SPTs with one of the allergen extracts tested, and titration of serum-specific IgE measured by RAST. These patients had either a clear-cut allergic sensitization or no evidence of allergy. Allergy to one or more of the allergens tested was characterized by a concordant history, SPTs, and RAST to the given allergen(s). Individuals with no evidence of allergic sensitization, negative SPTs, and RAST were classified as nonallergic to the given allergen(s). Patients with a doubtful allergy had been excluded from the study.

Extracts

A 9% codeine phosphate solution (Laboratoires des Stalergenes, Fresnes, France) was used as previously indicated.¹⁰

Standardized allergenic extracts were prepared by the Laboratoires des Stallergenes according to the proposals of the Allergen Subcommittee of the International Unions of Immunological Societies. The methods of preparation and control of pollen, mite, and cat extracts have been previously described in detail.¹⁰⁻¹³ All *Alternaria* extracts were standardized by in vivo (skin test titration) and in vitro methods (RAST inhibition, cross immunoelectrophoresis, immunoblotting, and isoelectric focusing).

Phazet is an allergen-coated lancet⁷ (Fig. 1). The amount of allergen on the tip of the Phazet needle has been calibrated against 100,000 BU, according to the Nordic concept on allergen standardization.^{14, 15} The allergen extracts (Pharmalgen, Pharmacia Diagnostics AB) used for coating Phazet needles were prepared according to the specifications of the Allergen Subcommittee of the International Unions of Immunological Societies. The methods for preparation and control of the extracts have previously been described.^{16, 17} All these extracts were standardized by in vivo (skin test titration) and in vitro methods (RAST inhibition, cross immunoelectrophoresis, immunoblotting, and isoelectric focusing). Phazet histamine was covered with histamine hydrochloride (10 mg/ml), and the strength was chosen as that inducing a positive response in all patients. No potency calibration was available.

Methods of SPTs

The modified SPT was performed according to the method of Pepys and Bernstein.³ A small drop of each test extract was placed on the surface of the skin. A disposable needle (3/8 inch; 25-gauge) was passed through the drop and inserted into the epidermal surface at a low angle with a bevel up, facing away from the surface. The needle tip was then gently lifted to elevate a small portion of the epidermis without inducing bleeding. The test solution was gently wiped away 1 to 2 minutes later.

The Phazet needle was inserted at 90 degrees to the skin surface. The point of the needle was pressed against the skin, applying the same pressure every time.

Tests

The entire study was performed by the same investigator between 9 and 12 hours to avoid circadian variations. None of the patients was taking any drug that might interfere with skin tests.¹¹ Skin tests were performed on the volar surface of the forearm. Test sites were placed 2 cm apart, as suggested by Bousquet¹ and Pepys.² Wheal sizes were recorded after 10 minutes by means of the cellophane tape technique, and mean diameters were averaged. With modified SPT, the test was considered to be positive when the wheal size was greater than that induced by codeine phosphate. Skin test with allergen-coated Phazet was considered positive when the wheal size induced by allergen was greater than that induced by histamine.

Design of the study

Each patient had an SPT by histamine Phazet and one or several SPTs by allergen Phazet, according to their sensitivities, and modified SPT with the same allergen species

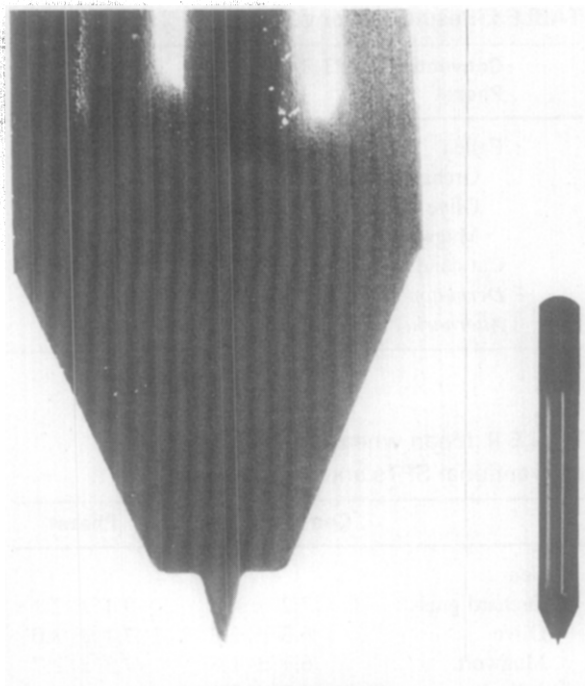


FIG. 1. The Phazet.

and codeine phosphate. For each allergen species, 20 to 30 nonsensitized patients were tested with Phazet and modified SPT. The definition of sensitivity (true positive in percent of patients with disease) and specificity (true negative in percent of patients without disease) was derived from Galen and Gambino.¹⁸

RESULTS

Results are presented in Tables I and II. There was no discrepancy between modified SPT and Phazet with orchard-grass and mugwort pollen, cat dander, and *Alternaria*. One patient had negative Phazet and a positive SPT RAST with olive pollens, and another patient had a positive Phazet with a negative SPT RAST with mites. The overall sensitivity of Phazet was 99.43%, and the overall specificity was 99.27%.

DISCUSSION

Phazet was found in this study to be a highly sensitive and specific puncture method because it correlated extremely well with SPT performed by a well-trained investigator using lyophilized standardized extracts freshly reconstituted.

The method of modified SPT favored by Pepys and Bernstein³ is widely accepted in Europe and is the reference technique when other SPT or puncture methods are being studied. In our clinic, this method has been used to perform trials on standardization of allergens, to assess the effects of various treatments, or to compare different SPT methods.² We routinely use codeine

TABLE I. Results of conventional SPTs and Phazet

Conventional SPT RAST Phazet	+	+	-	-
	+	-	-	+
Pollen				
Orchard grass	33	0	20	0
Olive	29	1	25	0
Mugwort	11	0	35	0
Cat dander	26	0	24	0
<i>Dermatophagoides</i>	36	0	14	1
<i>Alternaria</i>	40	0	20	0

TABLE II. Mean wheal sizes obtained with conventional SPTs and Phazet

	Conventional SPT	Phazet
Pollen		
Orchard grass	7.2 ± 4.5	9.1 ± 2.1
Olive	6.3 ± 2.3	7.1 ± 3.0
Mugwort	6.1 ± 1.8	7.3 ± 2.7
Cat dander	4.5 ± 2.1	6.1 ± 2.4
<i>Dermatophagoides</i>	4.7 ± 2.0	6.9 ± 3.1
<i>Alternaria</i>	4.3 ± 1.8	7.3 ± 2.1

Results are expressed in mean ± SD.

phosphate as a positive control solution. This mast cell secretagogue was chosen for the modified SPT method, whereas in the Nordic countries, histamine is the positive control favored. Therefore, we used two different control solutions.

The high correlation between the modified SPT and coated Phazet confirms previous investigations⁷⁻⁹ and indicates that the latter method may easily replace SPT and may eventually be superior (1) when unstandardized extracts are used or with extracts stored for a long period of time in which the potency is poorly characterized, (2) when untrained investigators perform skin tests, and (3) when young children are being tested in whom Phazet is easier to perform. Moreover, it is quicker than SPT, and the investigator is not in contact with allergen extracts. Therefore, Phazet appears to be safer for allergic nurses and doctors. The aim of this study was to assess the value of Phazet in characterized individuals, but the conclusions drawn may not be totally applied (1) to patients with equivocal sensitivities or (2) to subjects with a positive SPT and/or RAST without symptoms. Further studies are, however, required with provocative challenges to assess the value of both methods in these situations, although the potency of Phazet allergens has been chosen to discriminate between patients with clinical

sensitization and subjects without clinical sensitivities.^{12, 19}

The results were highly comparable with both techniques since standardized allergen extracts have been used. These correlations were expected for pollen and dander extracts, since they are now well characterized and standardized, but they were not expected for *Alternaria*, which is not yet completely standardized and in which extracts were found a few years ago to be highly variable.²⁰ The results of this study indicate therefore that the quality of allergen extracts has been seriously improved and that skin tests are even more reliable than in the past when skin tests were performed with unstandardized extracts.

REFERENCES

1. Bousquet J. In vivo methods for study of allergy: skin tests, techniques, and interpretation. In: Middleton E Jr, Reed CE, Ellis EF, et al., eds. Allergy: principles and practice, vol 1. 3rd ed. St. Louis: CV Mosby, 1988:419-36.
2. Pepys J. Skin testing. Br J Hosp Med 1975;14:412-7.
3. Pepys J, Bernstein IL. The modified skin prick test. XII International Congress of Allergology and Clinical Immunology. Washington, D.C.: 1985.
4. Voorhorst R, Van Krieken H. Atopic skin test reevaluated. II. Variability in results of skin testing done in octuplicate. Ann Allergy 1973;31:195-204.
5. Malling HJ. Diagnosis and immunotherapy of mould allergy. II. Reproducibility and relationship between skin sensitivity estimated by end point titration and histamine equivalent reaction using skin prick test and intradermal test. Allergy 1985;40:354-62.
6. Michel FB, Skassa-Brociek W, Segalen C, Dreborg S, Bousquet J. Reproducibility of six methods of skin prick tests [Abstracts]. J ALLERGY CLIN IMMUNOL 1986;77:222.
7. Belin L, Dreborg S, Einarsson R, Holgersson M, Halvorsen R, Lund B, Löfkvist T, Moxnes A, Ståhlenheim G, Wihl JA, Våla IJ. Phazet—a new type of skin prick test: calibration and stability. Allergy 1985;40(suppl 4):60-3.
8. Fälth-Magnusson K, Dreborg S, Kjellman NIM. Prick test with PHAZET™ compared with allergen extracts, serum IgE, and history in 314 adults [in press]. Allergy.
9. Holgersson M, Ståhlenheim G, Dreborg S. The precision of skin prick test with PHAZET™, the Østerballe needle, and the bifurcated needle. Allergy 1985;40(suppl 4):64-5.

10. Bousquet J, Djoukhar F, Hewitt B, Guérin B, Michel FB. Shelf life of a pollen and a mite standardized extract. *Clin Allergy* 1985;15:29-36.
11. Bousquet J, Calvayrac P, Guérin B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. *J ALLERGY CLIN IMMUNOL* 1985;76:734-45.
12. Didierlaurent A, Foglietti MJ, Guérin B, Hewitt B, Percheron F. Comparative study on cat allergens from fur and saliva. *Int Arch Appl Immunol* 1984;73:27-31.
13. Horst M, Bousquet J, Gouyon B, et al. Allergy to *Alternaria* [in press]. *Clin Allergy*.
14. Belin L. Biological standardization—the HEP unit. In: Sisk C, et al. Proceedings of the Fourth International Paul Ehrlich Seminar. Stuttgart: Gustav Fischer Verlag, 1987:145-53.
15. Dreborg S. The skin prick-test—methodological studies and clinical applications [Dissertation]. Linköping, Sweden: 1987.
16. Einarsson R, Dreborg S. Manufacturers criteria for in-house reference preparations. In: Sisk C, et al. Proceedings of the Fourth International Paul Ehrlich Seminar. Stuttgart: Gustav Fischer Verlag, 1987:131-8.
17. Uhlin T, Reuterby J, Einarsson R. Antigen/allergenic composition of poodle/alsatian dandruff extract. *Allergy* 1984;39:125-33.
18. Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnoses. New York: John Wiley, 1975.
19. Petersson G, Dreborg S, Ingestad R. Clinical history, skin prick test, and RAST in the diagnosis of birch and timothy pollinosis. *Allergy* 1986;41:398-407.
20. Argawal MK, Jones RT, Yunginger JW. Immunochemical and physicochemical characterization of commercial *Alternaria* extracts: a model for standardization of mold allergen extracts. *J ALLERGY CLIN IMMUNOL* 1982;70:432-6.

Antiallergic activity of H₁-receptor antagonists assessed by nasal challenge

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*Most oral drugs used for the treatment of allergic rhinitis are classified as H₁-receptor antagonists, and although they represent major sales throughout the world, their mechanism of action is still poorly known. In an attempt to understand better the in vivo therapeutic effects of these drugs, a double-blind, crossover study was carried out. The study compared the effects of terfenadine and loratadine, nonsedative H₁-receptor antagonists, on the immediate allergic response of the upper airways to challenge with orchard-grass pollens in 14 highly allergic subjects. Increasing numbers of pollen grains were insufflated into the nostrils, and the response of the subjects was assessed by examining symptoms and measuring the release of histamine and prostaglandin D₂ in nasal secretions. Each drug was administered for a week before challenge. This study demonstrated the clinical efficacy of both drugs by comparison to that of a control day, since symptoms were observed for a significantly ($p = 0.014$) greater number of pollen grains. Only one patient had a significant release of histamine when they were treated with loratadine versus 10 during control day ($p < 0.0023$) and six when they were treated with terfenadine ($p < 0.01$). Prostaglandin D₂ release occurred with a higher allergen dose when patients were treated with both drugs. This study indicates that some H₁ antagonists also possess antiallergic activities. (*J ALLERGY CLIN IMMUNOL* 1988;82:881-7.)*

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Histamine is one of the mediators of the nasal-allergic reaction, but recent studies in pollen-allergic individuals examining the release of mediators in nasal secretions during allergen challenge have suggested that lipid-derived mediators may also be relevant.¹⁻¹⁰ Moreover, the mechanism of action of H₁ antagonists may not only be antagonism of H₁ receptors,