

Standardization of the Prick Test

A Comparative Study of Three Methods

A. BASOMBA, A. SASTRE, A. PELÁEZ, A. ROMAR,
A. CAMPOS and A. GARCÍA-VILLALMANZO

Servicio de Alergia, Hospital La Fe, Valencia, Spain

The different needles and methods used in the prick test give rise to disparate results. This has significance when carrying out multi-centre studies and when using the technique in the standardization of allergenic extracts. With test reliability as our objective, prick tests were carried out on 30 subjects: 10 patients sensitive to *Dermatophagoides pteronyssinus* received a glycerine extract of known allergenic potency, 10 healthy individuals 2.5 % codeine phosphate in a glycerine solution, and another 10, histamine 1/1000. The total prick tests per individual was 27 with each of the needles employed (Allergy Pricker, the Morrow-Brown needle and Insulin needle in accordance with Pepy's procedure). The tests were carried out systematically by three different testers, and the total number of prick tests performed was 810. With the Allergy Pricker, no differences were observed among results obtained by the same tester, nor when the results of the three testers were compared. With the Morrow-Brown needle, the results varied in the same person and from one tester to another, and on many occasions the test was negative. With the Pepys method, no falsely negative results were obtained, but there was considerable variation in the size of the wheal. The variation coefficient is 41 % with the Allergy Pricker, and 115 % and 64 % with the Morrow-Brown and Pepys method, respectively. In conclusion, the results obtained clearly indicate that the highest degree of reproducibility is obtained with the Allergy Pricker.

Key words: reproducibility; skin prick test.

Accepted for publication 27 December 1984

The Prick Test (PT) is widely used to detect IgE-mediated hypersensitivity and, consequently, to diagnose atopic allergy. However, the multiplicity of methods does not allow an assessment of the results obtained by different work groups, and the differences are important when carrying out multi-centre studies and particularly when using the technique in the standardization of allergenic extracts.

The factors involved in the variability of results are the time interval and manner of taking readings, type of needle used, methodology and the experience of the person carrying out the test.

The objective of this paper is to analyse the differences between three PT methods, and to investigate the reproducibility of each of them.

MATERIAL AND METHODS

Thirty individuals aged between 15 and 56 years underwent PT with three types of needles. Ten of the subjects were known to be sensitive to *Dermatophagoides pteronyssinus* (positive skin test) and the remainder were members of our hospital staff, in apparent good health and non-allergic.

The needles were: the Allergy-Pricker (Dome/Hollister-Stier) (3), the new Morrow-Brown needle (1) and an Insulin needle, in accordance with the conventional method modified by Pepys (5, 6).

The substances used were:

Dermatophagoides pteronyssinus extract in glyce-

Table 1

Results obtained by three testers, using three different needles, when testing histamine, codeine and Dermatophagoides extract on 30 subjects

Test substance	Tester No.	Prick test method			Analysis of variance* F_{87}^2
		Allergy pricker	M. Brown	Pepys	
Histamine ($n = 10$)	1	$\bar{x} = 23.21$	2.65	15.16	58.9
		SD = 8.06	2.73	9.56	$P \ll 10^{-6}$
		$n = 30$	30	30	
	2	$\bar{x} = 21.93$	4.67	19.87	32.3
		SD = 9.81	4.57	11.42	$P \ll 10^{-6}$
		$n = 30$	30	30	
	3	$\bar{x} = 19.47$	3.98	15.06	27.5
		SD = 8.68	4.48	10.63	$P \ll 10^{-6}$
		$n = 30$	30	30	
	F_{87}^{2**}	1.48 n.s.	1.98 n.s.	2.03 n.s.	
Codeine ($n = 10$)	1	$\bar{x} = 28.75$	2.73	19.06	42.7
		SD = 9.71	4.53	15.79	$P \ll 10^{-6}$
		$n = 30$	30	30	
	2	$\bar{x} = 27.31$	4.58	29.96	61.9
		SD = 8.36	4.86	13.73	$P \ll 10^{-6}$
		$n = 30$	30	30	
	3	$\bar{x} = 26.71$	4.79	14.84	61.5
		SD = 9.85	5.59	6.91	$P \ll 10^{-6}$
		$n = 30$	30	30	
	F_{87}^{2**}	0.38 n.s.	1.53 n.s.	11.26 $P < 0.001$	
Dermatophagoides extract ($n = 10$)	1	$\bar{x} = 29.98$	3.97	22.59	51.5
		SD = 13.99	3.61	10.25	$P \ll 10^{-6}$
		$n = 30$	30	30	
	2	$\bar{x} = 30.62$	4.63	33.95	35.2
		SD = 14.67	5.29	20.37	$P \ll 10^{-6}$
		$n = 30$	30	30	
	3	$\bar{x} = 30.06$	6.18	21.11	23.2
		SD = 16.83	7.68	14.89	$P \ll 10^{-6}$
		$n = 30$	30	30	
	F_{87}^{2**}	0.01 n.s.	1.16 n.s.	5.98 $P < 0.01$	

$\bar{x}$ = arithmetic mean of the wheal areas in mm².

SD = standard deviation.

n = number of tests (3/patient).

* Fisher's test for the differences between the three PT methods.

** Analysis of variance (F) for the differences between the three testers.

rine solution, containing a known allergenic potency (expressed in conventional Biologic Units) produced by Abelló Laboratoies.

Histamine 1/1000 in glycerine solution.

Codeine phosphate 2.5 % in glycerine solution.

All the PTs were performed in the morning (from 10 a.m. to 12 a.m) for 3 successive days, and one substance per day was tested. The solutions were prepared immediately prior to the execution of the tests.

On the first day two testers with no previous experience of these tests (Nos. 1 and 3) and a highly experienced tester (No. 2) performed three separate PTs (with the 3 needles) on each of the 10 persons sensitive to *Dermatophagoides*, using the allergenic extract. The same procedure, using histamine on 10 subjects and codeine phosphate on the other 10 was performed on the second and third day, respectively.

Thus, the number of PTs carried out for each substance was 270 (27 on each individual: 3 testers $\times$ 3 PT $\times$ 3 needles) and the total number of PTs, in the whole study, was 810.

The PTs were carried out on the volar aspect of the forearm and none of the subjects tested had previously taken anti-histaminic preparations, ketotifen or any other drug.

The results were recorded by one of the testers by tracing the wheal contour with a fine fibre-tip pen and transferring the outline by means of 3M Pressure Sensitive Scotch Tape. The area was measured using the average of the largest and smallest diameters.

In a previous study, it has been established that the histamine results (maximal size of the wheal reaction) were best recorded after 10-12 min, while those with codeine phosphate and *Dermatophagoides* extract were best after 18-20 min. For this reason, the result of the histamine reaction was taken after 12 min and that of codeine and *Dermatophagoides* after 20 min.

For the statistical analysis, Student's *t* test was applied in order to study the differences obtained with the different solutions, needles and testers. Fisher's test was used to study the homogeneity of the averages, and the variance coefficient to study the reproducibility of the different methods.

RESULTS

Table 1 shows the results (mean and standard deviation of the wheal size) obtained by the three testers, when histamine, codeine and *Dermatophagoides* extract were prick tested with the three needles. The mean of the reactions obtained with each solution and for the same tester is significantly different according to the needle used: from a maximum mean area of 32 mm² with the Pepys method using *Dermatophagoides* extract by tester No. 2, to an average of 2.6 mm² obtained by tester No. 1 using the Morrow-Brown needle to test histamine. The analysis of variance *inter-PT-methods* is highly significant ($P \leq 10^{-6}$ in all the cases).

It was also observed that there are no significant differences attributable to the techni-

Table 2

The variation coefficient of the results obtained when performing the prick test with three different needles, with histamine, codeine and *Dermatophagoides* extract, carried out by the three testers

Test substance	Prick test method		
	Allergy Pricker	M. Brown	Pepys
Histamine			
$\bar{x}$	21.53	3.76	16.69
SD	8.91	4.06	10.68
<i>n</i>	90	90	90
% var	41.4	107	64
Codeine			
$\bar{x}$	27.59	4.12	21.28
SD	9.26	4.96	14.11
<i>n</i>	90	90	90
% var	33.5	120	66.3
Dermatophagoides			
$\bar{x}$	30.22	4.92	25.88
SD	15.04	5.78	16.58
<i>n</i>	90	90	90
% var	49.7	117	64
Mean of variation coefficient	41.5	115.2	64.7

$\bar{x}$ = arithmetic mean of the wheal areas in mm².

SD = standard deviation.

n = number of tests.

% var = variation coefficient.

cian or tester when using the Allergy Pricker or the Morrow-Brown needle: the analysis of the variance *inter-testers* shows no difference in either case. The wheal size is very small when the Morrow-Brown needle is used (between an average of 2.65 mm² obtained by tester 1 with histamine and 6.18 mm² by tester 3, with *Dermatophagoides*). In 28.2 % of the PTs carried out with this needle, there was no reaction at all (no wheal).

With the Pepys method, however, there was a significant difference in the results obtained by the different testers when using codeine or *Dermatophagoides* ($P < 0.001$ and 0.01, respectively) but not with histamine. Tester No. 2 (experienced) obtained greater reactions than the other two with this method.

Table 2 shows the variation coefficient (arithmetic mean/SD), which is 41 % with the Allergy Pricker and 115 % and 64 %, respectively, with the Morrow-Brown and Pepys method, for the three testers as a whole. This shows the *inter-tester* precision.

DISCUSSION AND CONCLUSIONS

At present, the prick test is the most efficient technique in the detection of immediate hypersensitivity. During recent years, methods and the needles used have multiplied, in particular: Pepys (5, 6), Morrow-Brown (1), Østerballe (7), Nierop (4), the Allergy Pricker (3), the Lincoln Multitest (2). Such a variety gives rise to disparate results in multi-centre studies, which do not take this fact into account, and becomes more important when standardizing allergens.

In our studies we have used three types of lancets and methods. The Allergy Pricker (3) is a precision needle standardized by Dome/Hollister Stier, with a point length of 1 mm, and an inclusive angle of 25°. This needle is very similar to the one recommended by Østerballe (7).

The Morrow-Brown needle is available in disposable plastic (though a stainless steel version is also available), with a point length of 1.0 mm and an inclusive angle of 25°. The lancet measures 3 cm and is 3 mm wide. The point length is pyramidal in shape. The skin penetration angle for both needles is 90°. The Pepys

old system, uses a disposable hypodermic needle, pricked into the skin at a penetration angle of 60° to 80°.

In our research we have simply tried to verify the reproducibility, or *inter-tester* precision, of each of the three methods. The results obtained indicate that the highest degree of reproducibility (i.e. the lowest variation coefficient) with any of the substances used (histamine, codeine or *Dermatophagoides* extract) is obtained with the Allergy Pricker, followed by the Pepys method, and the least reproducible method is that of Morrow-Brown. The *inter-tester* precision is of the same order as the *intra-tester* precision (variation coefficient for the results of the single tester considered).

The results obtained with the Allergy Pricker and the Morrow-Brown needle do not vary in accordance with the expertise of the operator. However, when using Pepys' classical method, the differences become highly significant in relation to whether or not the tester is familiar with the technique. Finally, with the Morrow-Brown needle, the number of negative tests is very high, with whatever tester (experienced or non-experienced) and whatever substance, and in the positive cases the wheal size does not usually exceed 5 mm².

The variability of the results with the Pepys method according to the experience of the tester depends basically on the difference in the pressure exerted, the degree of needle penetration and its angle of application.

With the Morrow-Brown needle, correct penetration of the skin is not obtained when pressure is applied due to the pyramidal-shaped point and the shape or size of the base, which is circular. The concavity of the skin is increased as a result of the circular pressure. The Allergy Pricker, with a point of 1 mm, has a laminar section the base of which exerts the right amount of pressure to prevent greater needle penetration, but does not produce the concavity that prevents adequate penetration of the point. A similar needle has been standardized and the reproducibility of the method has been studied by Østerballe (7) who has obtained the best results with the 1 mm point as opposed to other size types (from 0.6 to 1.3 mm).

The results obtained in the present study lead us to conclude that the Allergy Pricker is a good needle for the prick test, offering greater reproducibility and uniformity of results, i.e. greater *intra* and *inter-tester* precision. The execution is straightforward and the results homogeneous whatever the substance tested. At its best, the coefficient of variance for the Allergy Pricker, is between 30 and 50 %.

ACKNOWLEDGEMENT

Our thanks are given to Miss Maria Dolores Baixauli for her excellent technical assistance.

REFERENCES

1. Brown, H. N., Su, S. & Thantrey, N.: Prick testing for allergens standardized by using a precision needle. *Clin. Allergy* *11*, 95-98, 1981.
2. Knoker, W. T., Anderson, C. T. & Roumiantzeff, M.: The multi-test system: a standardized approach to evaluation of delayed hypersensitivity and cell-mediated immunity. *Ann. Allergy* *43*, 73-79, 1979.
3. Malling, H. J., Andersen, C. E., Boas, M. B., Holgersen, F., Munch, E. P. & Weeke, B.: The Allergy Pricker. Qualitative aspects of skin testing using a precision needle. *Allergy* *37*, 563-567, 1982.
4. Nierop, G., Voorhorst, R. & Temmerman-Van de Vijver, R. L.: Atopic skin test re-evaluated. X. Comparison of a perfected technique of intracutaneous skin testing and the prick performed with a modified Morrow-Brown needle. *Ann. Allergy* *46*, 105-109, 1981.
5. Pepys, J.: Skin tests in diagnosis. In Geel, P. G. H. & Coombs, R. R. A. (eds.): *Clinical aspects of immunology*, 2nd ed., p. 192. Blackwell Scientific, Oxford, 1968.
6. Pepys, J.: Skin testing. *Br. J. Hosp. Med.* *14*, 412, 1975.
7. Østerballe, O. & Weeke, B.: A new lancet for skin prick testing. *Allergy* *34*, 209-212, 1979.

Address:
Dr. Antonio Basomba
Servicio de Alergia
Hospital La Fe
Valencia
Spain

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.