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Some Variables in Skin Prick Testing

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Skin prick testing (SPT) represents an indispensable tool for the routine diagnostic work in allergy. It is the skin test method which has the highest degree of diagnostic specificity provided satisfactory allergen preparations are used (2,4,7,10). SPT is considered also to be the most lenient of the different skin test techniques. Only minimal amounts of the actual allergen are introduced into the skin during SPT, and the technique implies very small risks for serious reactions (3, 5, 7, 8).

For SPT to be applicable as bio-assay in scientific work and in standardized procedures, the variables of the method must be known and controlled. For this purpose, we have performed several studies, many of

them unpublished, in addition to borrowing data from others.

Histamine appears to be the mediator which determines the local allergic reaction to skin testing, and is well suited as reference (3,9,12). Histamine hydrochloride 1 mg/ml (histamine 5.43 mmol/l) has been established as the standard reference for skin prick testing by the Northern (Scandinavian) Society for Allergy (1,12).

There is some diversity in the patient population as regards the reactivity to histamine in addition to the wide distribution of immunological reactivity to various allergens. The use of a given histamine reference com-

pensates for the former variable. No method of reference can compensate for the latter variable, since it is inherent in biology itself. However, with use of biologically standardized material and the histamine reference together, more meaningful information is obtained from the size of the wheals obtained (1). An extract adjusted to 1 Histamine Equivalent potency in SPT elicits a skin reaction equivalent to that of the histamine reference in the majority of the patients who represent the average population allergic to the allergen in question. A stronger reaction indicates that the patient tested has more than average specific IgE on the skin mast cells. A weaker reaction indicates that the patient is less than average skin sensitive to the particular allergens.

The given concentration (1 mg/ml) has been found optimal for us and our associates since it elicits wheal reactions in the order of 6–8 mm in most patients with our way of performing SPT (1,3,12).

A reaction (the wheal) of the same order of size as that of the histamine reference is recorded as three plusses (+ + +). Reactions to SPT with allergens corresponding to + + (50% of the + + + reaction wheal) are usually immunologically specific. In contrast to the experiences of Voorhorst (11) we find the flare reaction to be too variable in the same individual to be relied on, and we record only the wheal. Any problem of time differences for the maximum of response to histamine and allergen, respectively, as reported by Voorhorst, is easily solved by simply outlining the wheal to the SPT with histamine after 10 min, using a soft ball-point pen, and then using this as reference for the allergen SPT reaction when the latter is recorded after 15–20 min.

The way of performing SPT is, however, an important variable. In one small (unpublished) study, two persons experienced in SPT simultaneously performed an extended series of SPT with various concentrations of histamine on the same patient. One person had to use 10 times stronger histamine concentrations than the other to elicit whealing reactions of the same size. This has been confirmed in other unpublished studies, and by reports from centers using histamine 1 mg/ml or 10 mg/ml, respectively, as reference. A main function of the use of histamine as reference is to detect the influence of this kind of technique variability and to compensate for it. It also allows the test performer to adjust the technique to achieve optimal results within the frames of standardization.

Another important variable is the degree of reproducibility or repeatability of the test as performed by each test performer repeatedly on the same or different individual, on the same day or on different occasions. We have estimated this by performing a large number of SPT with the same material on the same individual. Some testers have been shown to perform the test with a reproducibility regularly within a $\pm 10\%$ variation from the mean, although not in 100% of all tests done. Others may show regular variations of more than $\pm 30\%$ from the

mean. In any case, everyone who performs a large number of SPT apparently must be prepared to obtain one or another test result which is not satisfactory as to repeatability, although a remarkable repeatability can be achieved.

An outfit which makes identical SPT lesions for each application might possibly facilitate the repeatability of the test. In order to study this variable, two different types of outfit, designed particularly to obtain standardized puncture lesions in skin prick testing, were compared with our routine outfit, which makes use of a short, bevelled disposable injection needle:

- A disposable short-bevelled needle used for routine clinical practice in our department.
- A special SPT standard needle designed by Morrow Brown (6).
- A Multi-test applicator which allows eight puncture tests to be performed simultaneously.

To eliminate other variables as much as possible, in all tests performed the histamine reference material was applied by one medical nurse with long experience in performing the SPT. All tests were performed on one and the same volunteer on different occasions with intervals of 1–4 weeks. All tests were performed on the volar aspects of the forearms. Great care was taken to elicit even pressures on all three types of outfit during all tests, and careful efforts were made to achieve reproducible results. A total of 80 SPTs were performed with outfits A and B in parallel and with the B and C outfits in parallel (Table 1).

The A and B outfits produced quite similar results with a very high degree of repeatability. They were easy to use, but since the B outfit makes demands as to cleaning and sterilization, it appeared quite impractical for us compared with the disposable A outfit. The Multi-test outfit had no advantage over the other two types of outfit. On the contrary, it produced less reproducible results than the other ones unless only one Multi-test applicator was used on the middle of the forearm only. Then the reproducibility was of the same order as that obtained by the two other types of outfit.

In our hands a quite wide range of needles and lancets may be used in SPT without much effect on the size of reactions obtained. The latter seems to depend

Table 1

Outfit	No. of SPT	$\frac{D+d}{2}$ mm		V*	Range
		mean	s.d.		
A	80	6.1	0.51	8	5–7
B	80	5.7	0.55	10	4.5–7
C	80	4.5	1.35	30	2–7

* Coefficient of variance (%).

more on how the outfit is used. In any case, differences in results which are due to outfit characteristics are compensated for when reactions to allergens are compared with reactions to the histamine reference. This is important, since it may be next to impossible for some testers to purchase the same kind of needle or lancet as others.

Clinicians entering immunological or protein chemistry laboratories are frequently quite impressed by the seemingly high degree of precision suggested by instruments indicating the readings in red numbers. As a matter of fact, the skin test technique can be as precise as many of the *in vitro* tests in question. In the laboratory it is mandatory to control the reliability of assay instruments as to precision and reproducibility. Standard reference material is used for this purpose, and the deflection of the indicators is adjusted to optimize precision of the readings. I would like to emphasize the need to apply similar principles to biological techniques. For the SPT much can be achieved simply by:

1. Inclusion of histamine 5.43 mmol/l as reference.
2. Adjusting the personal technique to achieve wheal reactions to the histamine reference with mean diameters $\left(\frac{D+d}{2}\right)$ within the 5–8 mm range.
3. Applying a technique which gives reproducible results.

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