

Prick testing for allergens standardized by using a precision needle

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Summary

A new standardized precision needle which virtually removes operator variation from skin prick testing with allergens is described. The instrument is simple and robust, and the results of its use are demonstrated. The most important finding was that the use of strong pollen extracts produced very inconsistent and irregular weals, but when suitably diluted, or used on mildly sensitive skins, reproducibility of the response was excellent and it is therefore ideal for titration of skin sensitivity and biological assessment of the potency of allergens. The standard needle was also compared with two other methods of skin prick testing. Results were similar but the standard needle showed the least variation.

Introduction

Skin-test reactions to allergens must depend on the method of testing, the potency and purity of the allergenic extract, and on the sensitivity of the skin. The amounts of allergen introduced into the cutis vera by the prick, scratch, or intra-dermal method can vary greatly, thus causing poor reproducibility. Recent investigations of these methods (Indrajana *et al.*, 1971) and a review of previous research (Voorhorst & Van Krieken, 1973a,b) concluded that an intradermal method requiring special syringes and much skill was the most reproducible, and the prick test was the method most subject to variation.

Aas and colleagues (Aas & Belin, 1972, 1974; Aas *et al.*, 1978) proposed that allergenic extracts be standardized by the combined use of the skin prick test and the radioallergosorbent test (RAST). The prick test reaction to histamine hydrochloride (1 mg/ml) would be the reference standard for biological activity, and the concentration of allergen which produced an identical weal would be designated as one 'histamine equivalent prick' (1 HEP) potency, also denoted as 1000 biological units (BU)/ml. However, it was necessary to acquire skill to obtain prick test reactions in ten patients

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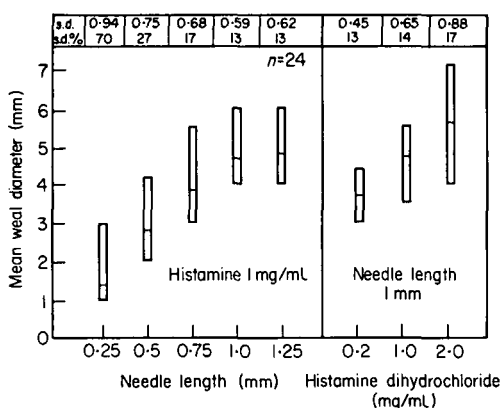


Fig. 1. Results of variation of needle length and of histamine concentration in twenty-four patients.

which did not vary more than $\pm 20\%$ from the mean, and large panels of volunteer patients, sensitive to allergens, were required, as well as RAST inhibition tests (Aas *et al.*, 1978).

The prick test is simple, easy and safe, but previous methods lack precision. It was apparent that the usefulness of the test could be enhanced if the injury to the skin, and thus the amount of allergen entering the cutis vera, could be standardized and operator skill eliminated.

Materials and methods

An adjustable needle was constructed to establish the optimum penetration which would produce a skin reaction without bleeding. In use the needle is jabbed into the skin at right angles, avoiding any lateral movement which would make a scratch, and the test material is not wiped off.

Drops of histamine hydrochloride (1 mg/ml) were placed on the skin of the back, 5 cm apart, at room temperature. The needle was jabbed through the drops, using different needle projections which were coded until the weals had developed and could be outlined with a fine Biro pen and transferred to paper. The weals were measured by taking the mean of the greatest diameter and of the diameter at right angles to its mid-point.

The needle length of 1 mm was selected as optimum because the 1.25 mm penetration often caused bleeding and the weals were not significantly larger (Fig. 1). Further tests using three coded dilutions of histamine were carried out (Fig. 1), and, as the gradation of results was satisfactory, large numbers of needles were produced in stainless steel as shown (Fig. 2). The end of the rod prevents penetration beyond 1 mm, and the point will hold a tiny drop of testing material. Tests for reproducibility, and to show any variation in skin sensitivity on different areas of the back, were carried out in four or five rows of four, equally spaced across the back, using pollen extract or histamine.

The new standard needle was also compared with two other methods, using three 10-fold dilutions of Dome Laboratories' grass pollen extract, 10 000 PNU, on pollen-sensitive patients. These were the modified prick test method of Pepys (1968, 1975), which involves directing a sterile disposable hypodermic needle 40 mm long by 0.8 mm diameter through a drop of test material on the skin at such an angle as to lift up the

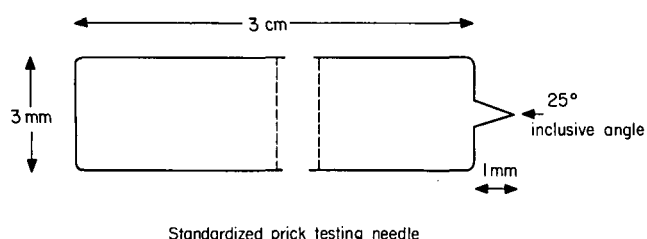


Fig. 2. The final design of precision needle made in stainless steel.

skin and make a microscopic channel through which the histamine or the extract can penetrate, and the original prick test method of Squire (1950) using a straight Hagedorn-type surgical cutting needle about 5 cm long. The non-cutting part of the needle is held between the forefinger and thumb so that friction gives fine control of the degree of penetration when the needle is plunged through the drop of test material into the skin, avoiding bleeding.

Results

Using histamine with the precision needle gave remarkably reproducible skin-test reactions at all strengths. However, using strong grass-pollen extracts, all three methods produced large weals so irregular in size and shape that it was impossible to measure them accurately. With more dilute extracts producing smaller weals the variation became less, until the reactions became very similar to those produced by 1 mg/ml histamine hydrochloride. Thus a concentration of 100 protein nitrogen units of five grass pollen (Dome) seems roughly equivalent to 1 HEP (1000 BU), in most pollen-sensitive patients.

Comparison of the three methods of prick testing demonstrated clearly that the Pepys method showed the greatest scatter, followed by the Hagedorn needle method, but the precision needle method is by far the most reproducible. (Fig 3). However, the scatter in weal size decreases sharply with lower concentrations of extract until, for 100 PNU, there is little difference between the three methods, especially if allowance is made for the greater errors in measurement inevitable for weals of less than 5 mm in diameter.

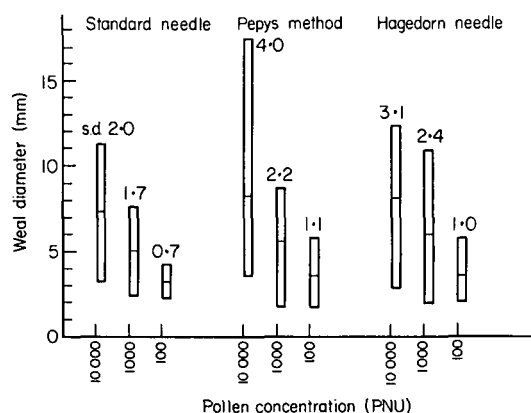


Fig. 3. Comparison of three methods of prick testing in twenty-five patients.

Discussion

The investigations of Idrajana, Spieksma & Voorhorst (1971) and Voorhorst & Van Krieken (1973a,b), comparing crude prick tests using a disposable lancet with very accurate intradermal tests, found unacceptable scatter in the prick method, but a range of lower concentrations was not used. Our findings that even when skin trauma is standardized, the strong extracts cause large irregular weals, but with weak test material the variation is relatively slight whatever method is used, seem important in relation to biological standardization, using the HEP method.

A modified disposable lancet for prick testing also with an optimum length of 1 mm, has recently been suggested (Østerballe & Weeke, 1979). However, the percentage standard deviation for weal size obtained with both histamine and pollen of 1 HEP potency was greater with the new lancet than with a crude lancet similar to that used by Voorhorst and his colleagues. No details of the precise dimensions of this lancet, except the length of the point, are given and it is obvious that this type of instrument, punched out from metal strip, has a chisel point, causing a cut rather than a prick. All tests with this lancet were conducted using 1 mg/ml histamine or grass pollen of 1 HEP potency, so that differences in weal sizes would be minimal and would give a false idea of the precision of the lancet.

The standard precision needle we have described has been used by us for the last 5 years. It is not difficult to manufacture, is easily sterilized and is robust in use.* It has also been found most helpful for titration of sensitivities, as we have recently described (Brown, Thantrey & Jackson, 1979).

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* A plastic disposable needle of the same design is now available through this Research Association, as they are now being made for us by Pressac Ltd, Acton Grove, Long Eaton, Nottingham.

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