

# Reproducibility of Histamine Skin Prick Test

Inter- and Intravariation Using Histamine Dihydrochloride 1, 5, and 10 mg/ml

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The reproducibility of skin prick test using histamine dihydrochloride 1, 5, and 10 mg/ml was tested by three nurses in five non-atopics in a double-blind trial. The variations day-to-day, within-day, between and for the same tester were calculated. Seventy-five percent of wheal reactions obtained by histamine 1 mg/ml were  $< 15 \text{ mm}^2$ . With histamine 5 mg/ml there were only a few wheals  $< 15 \text{ mm}^2$  and none at all with histamine 10 mg/ml. The mean coefficient of variation of wheals  $> 15 \text{ mm}^2$  was between 20-30%, in contrast to figures between 30-60% with wheals  $< 15 \text{ mm}^2$ . No significant day-to-day or within-day variation was shown concerning histamine wheal areas. It is suggested that histamine dihydrochloride 10 mg/ml should replace histamine dihydrochloride 1 mg/ml as the positive reference in routine skin prick tests and biological standardization.

*Key words:* histamine; reproducibility; skin prick test.

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## CLINICAL ASPECTS

**The reproducibility using 1, 5, and 10 mg/ml histamine dihydrochloride for skin prick test was assayed in a double-blind trial. It is concluded that histamine dihydrochloride 10 mg/ml should replace histamine dihydrochloride 1 mg/ml as positive reference when performing skin prick tests.**

Biological standardization of allergen extracts has been intensively discussed (1, 3, 14, 16). The Nordic guidelines (17) recommend the use of histamine dihydrochloride 1 mg/ml as the positive reference when performing skin prick tests (SPT). It is obvious that accurate biological standardization with SPT requires that at least the positive references have a high degree of reproducibility. Recently, it has been shown that "the Allergy Pricker" (a standardized SPT needle (6)) is superior to a standard blood lancet as regards reproducibility of the wheal area. The reproducibility of SPT using hist-

amine dihydrochloride 1, 5 and 10 mg/ml was examined in this double-blind trial.

## MATERIAL AND METHODS

### *Test persons*

Five, healthy, non-allergics (4 males and 1 female) mean age 34 (range 32-37 years) were included in the first part of the study. In the second part, one of the five test persons (a male, aged 36) was not available and a female, aged 36 was included. None of the test persons

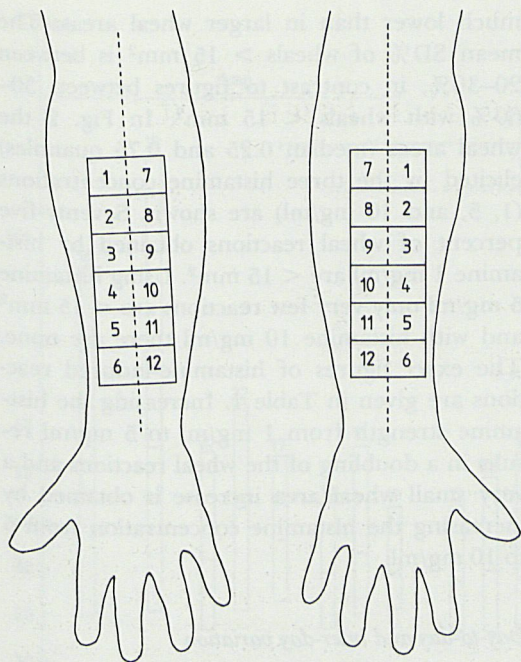


Fig. 1. Experimental design. Skin prick test with histamine dihydrochloride 1, 5, and 10 mg/ml, randomized in vials labelled 1–12, applied on corresponding sites on both forearms.

took any medication at least 2 weeks before the SPT.

#### Extracts

Histamine dihydrochloride 1, 5, and 10 mg/ml dispensed in 50% glycerol saline (Allergologisk Laboratorium A/S, Copenhagen, Denmark) was used. The histamine solutions were stored at +4°C when not in use.

#### Skin prick test

A standardized 1 mm precision needle – “the Allergy Pricker” (6) – was used. The allergy pricker was jabbed through the drops at an angle of 90° to the skin with moderate force and removed, and the drops wiped off. Wheal reactions were recorded after 12 min. The contour of the wheal area was outlined using a fine fibre-tip pen and transferred to a record sheet by means of tape. The wheal area was calculated

using electronic planimetry (Hewlett-Packard 9111 A graphic tablet interphased to a Hewlett-Packard 85).

#### Experimental design

All SPTs were carried out by the same three test nurses throughout the study.

*First part.* Each nurse skin prick tested the five test persons between 8 and 10 a.m. at days 0, 1, 3, 7. At day 7 tests were also carried out at 11–12 a.m. and at 3–4 p.m. with at least 3 h interval. To avoid interference from the previous SPT, the SPTs at day 7 were not performed on the previous site. After the six SPT sessions within 1 week, a free interval of 3 weeks was allowed to elapse before the next test session. The histamine concentrations 1, 5, and 10 mg/ml were labelled 1–12 (4 bottles of each strength) in random order, and at each session were applied in two rows (6 in each) to the volar side of the forearms (Fig. 1). SPT was carried out 5 cm below the elbow flexure to 5 cm above the wrist at intervals of 3 cm.

*Second part.* After 10 months the five persons were skin prick tested between 8–10 a.m. with a 1-week interval between tests performed by the three test nurses. Twelve bottles of histamine dihydrochloride 5 mg/ml were labelled 1–12 and the concentration applied as in the first part of the study. The study was double-blind and the code was not broken until all wheal areas had been measured.

#### Statistics

The reproducibility (coefficient of variation = SD%) was calculated as standard deviation divided by the arithmetic mean  $\times 100$ . Low values of SD% represent a high reproducibility and vice versa.

Differences in reproducibility between the three testers were evaluated with a two-tailed t-test. Analysis of variance was applied to evaluate systematic changes in wheal areas in each person during 1 week and during 8 h. A Wilcoxon's signed rank test was used to test differ-

ence in wheal area between left and right arm and between proximal and distal part of the forearm. The same test was used to evaluate changes of wheal area induced by histamine 5 mg/ml within a 10-month period.

## RESULTS

The presented data are based on a total of 2,520 skin prick tests.

### *Reproducibility*

The reproducibility (SD%) in relation to the wheal area (given in intervals of 5 mm<sup>2</sup>) for test nurses I, II, and III is recorded in Fig. 2. Two points are evident: there is no significant difference between the three testers, and the reproducibility of wheal reactions < 15 mm<sup>2</sup> is

much lower than in larger wheal areas. The mean SD% of wheals > 15 mm<sup>2</sup> is between 20–30%, in contrast to figures between 30–60% with wheals < 15 mm<sup>2</sup>. In Fig. 2 the wheal areas (median 0.25 and 0.75 quantiles) elicited by the three histamine concentrations (1, 5, and 10 mg/ml) are shown. Seventy-five percent of wheal reactions obtained by histamine 1 mg/ml are < 15 mm<sup>2</sup>. Using histamine 5 mg/ml only very few reactions are < 15 mm<sup>2</sup> and with histamine 10 mg/ml there are none. The exact figures of histamine-induced reactions are given in Table 1. Increasing the histamine strength from 1 mg/ml to 5 mg/ml results in a doubling of the wheal reactions and a very small wheal area increase is obtained by increasing the histamine concentration from 5 to 10 mg/ml.

### *Day-to-day and inter-day variation*

Two-way analysis of variance showed no significant day-to-day-variation of wheal area as tested by the three nurses using three histamine concentrations (Table 2). However, a significant inter-tester variation was found. Tester II induced significantly larger wheal reactions than those of I and III (Table 2).

Test nurse I compared with II, and II compared with III produced significantly different wheal areas with histamine chloride 1 mg ( $P =$

Table 1

The overall wheal area mean, median, range, and 25–75% in mm<sup>2</sup> for histamine dihydrochloride 1, 5, and 10 mg (based on all SPTs in the first part of the study, data from Table 2)

| Histamine dihydrochloride | Mean | Median | Range | Percentile 25–75 % |
|---------------------------|------|--------|-------|--------------------|
| 1 mg                      | 11   | 12     | 4–19  | 8–15               |
| 5 mg                      | 22   | 24     | 14–30 | 18–27              |
| 10 mg                     | 27   | 25     | 16–34 | 24–32              |

Table 2

Mean area for all six test sessions in mm<sup>2</sup> for each tested person (P<sub>1</sub>–P<sub>5</sub>) in relation to histamine concentration and test nurse (I, II, and III)

| Histamine dihydrochloride | Test nurse | Mean area for all 6 test sessions (mm <sup>2</sup> ) |                |                |                |                |
|---------------------------|------------|------------------------------------------------------|----------------|----------------|----------------|----------------|
|                           |            | P <sub>1</sub>                                       | P <sub>2</sub> | P <sub>3</sub> | P <sub>4</sub> | P <sub>5</sub> |
| 1 mg                      | I          | 8                                                    | 15             | 12             | 9              | 5              |
|                           | II         | 11                                                   | 15             | 19             | 14             | 12             |
|                           | III        | 5                                                    | 16             | 14             | 8              | 6              |
| 5 mg                      | I          | 19                                                   | 30             | 24             | 22             | 14             |
|                           | II         | 24                                                   | 28             | 30             | 25             | 21             |
|                           | III        | 14                                                   | 27             | 26             | 18             | 15             |
| 10 mg                     | I          | 24                                                   | 34             | 29             | 25             | 18             |
|                           | II         | 25                                                   | 32             | 34             | 31             | 25             |
|                           | III        | 16                                                   | 34             | 32             | 24             | 18             |

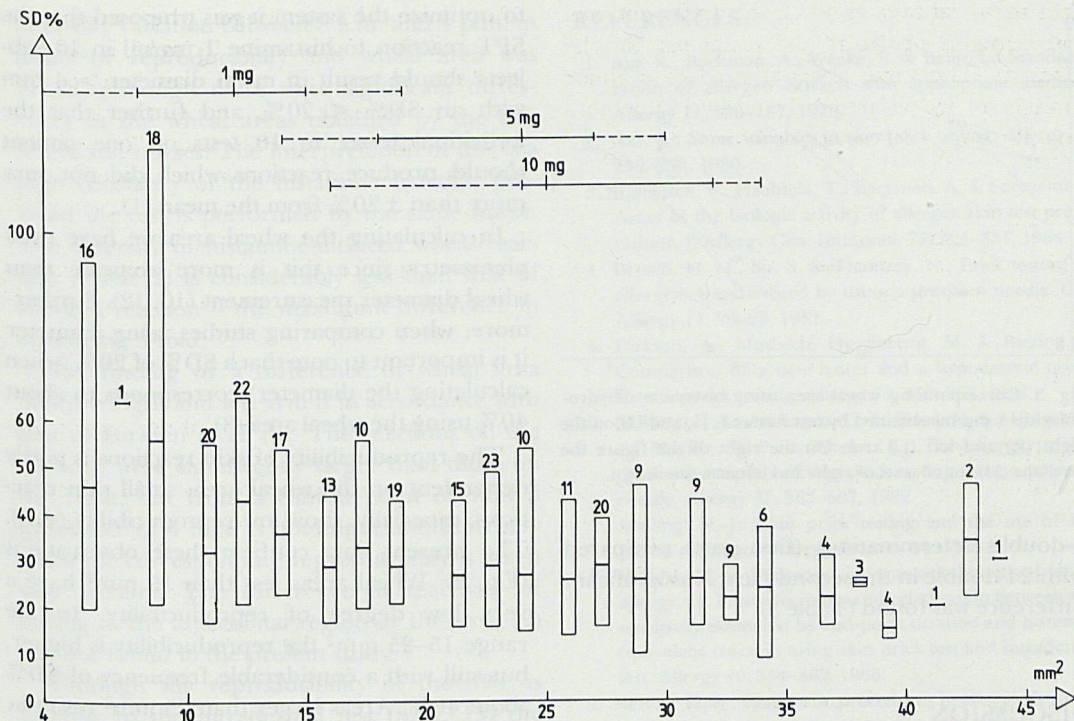


Fig. 2. Coefficient of variation (SD%) for test nurses I, II, and III, given as a function of the corresponding wheal area in mm<sup>2</sup>. SD % calculations are based on 8-double tests. The number of tests in each area interval is indicated on top of the columns, and the columns in each interval represent testers I, II, and III, respectively. Mean and range are outlined in each column.

At the top median, 25–75 % percentile and range of the wheal area obtained by histamine dihydrochloride 1, 5, and 10 mg/ml are outlined.

0.014 and  $P = 0.016$  respectively) and 5 mg ( $P = 0.038$  and  $P = 0.012$  respectively). With histamine 10 mg/ml only tester II compared with III differed significantly ( $P = 0.042$ ). Furthermore, no inter-day variation based on the three SPT sessions on day 7 was found (data not shown).

#### *Wheal area difference between right and left arm*

Corresponding mean areas of histamine 5 mg/ml wheals on right and left arm (12 double determinations on each arm) are plotted in Fig. 3. In all but two of 15 test sessions were larger areas elicited on the left arm. The difference is significant ( $P < 0.01$ ), but the median difference is small: from 19 mm<sup>2</sup> (right) to 23 mm<sup>2</sup> (left).

#### *Wheal area difference between proximal and distal part of forearm*

To systemically investigate wheal area differences between the proximal and the distal part of the forearm, the results obtained with histamine 5 mg/ml were evaluated (the proximal 4 SPT wheal areas of both arms compared with the distal 4). No significant difference was found.

#### *Wheal area difference over a 10-month period*

To evaluate the constancy of histamine-induced wheal reactions over a 10-month period the mean wheal area of histamine 5 mg/ml obtained at day 1 (first part of study) was compared with the results obtained in the second part of the study. Only four persons were tested and the data based on

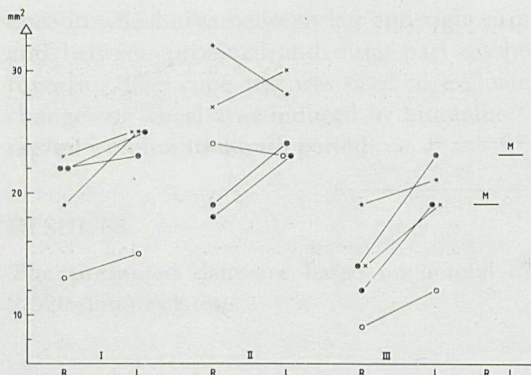


Fig. 3. Corresponding wheal area using histamine dihydrochloride 5 mg/ml obtained by test nurses I, II, and III on the right (R) and left (L) arm. On the right of the figure the medians (M) for all tests of right and left arm are shown.

8-double determinations (first part) compared with 24-double in the second part. No significant difference was found (Table 3).

## DISCUSSION

The use of histamine as positive reference in evaluating the skin response is generally accepted in Scandinavia (1). A crucial point in assessing the value of the skin test as a diagnostic tool is the reproducibility, and especially the reproducibility of the reference. In order

to optimize the system it was proposed that the SPT reaction to histamine 1 mg/ml in 10 subjects should result in mean diameter  $> 4$  mm with an SD%  $< 20\%$ , and further that the individual tester in 10 tests on one patient should produce reactions which did not vary more than  $\pm 20\%$  from the mean (1).

In calculating the wheal area we have used planimetry since this is more accurate than wheal diameter measurement (10, 12). Furthermore, when comparing studies using diameter it is important to note that a SD% of 20% (when calculating the diameter) corresponds to about 40% using the wheal area (8).

The reproducibility of skin reactions is partly dependent on the wheal area, small skin reactions, especially, show low reproducibility (4, 8). The present data confirm these observations (Fig. 2). Wheal areas less than  $15 \text{ mm}^2$  have a very low degree of reproducibility. In the range  $15\text{--}25 \text{ mm}^2$  the reproducibility is higher, but still with a considerable frequency of SD% about 40%. Areas larger than  $25 \text{ mm}^2$  result in very few SD% about 40%.

The presented data of reproducibility are in accordance with those previously published (4, 6, 11, 15) as also the relation to the size of the wheals (4, 6, 8). The skin prick device used and the experience of the tester are of crucial importance (2, 4, 5, 6). In the present study "the Allergy Pricker" was used, and has been shown to give more reproducible results than an ordinary blood lancet (6). All test nurses were experienced, a point strengthened by the fact that the reproducibility did not change over a 10-month period.

The relationship between reproducibility, wheal area, and histamine concentration is important in the choice of a positive reference. Our data confirm previous reports of the inadequacy of using histamine 1 mg/ml as reference due to the small wheal area (6, 7, 8). To obtain wheal areas larger than  $15 \text{ mm}^2$  and a higher reproducibility, histamine 10 mg/ml is required.

The reproducibility based on daily tests during 1 week and repeated after 10 months did not change significantly, which also was the case with the absolute wheal area. Furthermore, no

Table 3

Mean areas of 5 mg histamine dihydrochloride at day 1 first part (start) and second part (end)

| Test person    |       | Test nurse |    |     |
|----------------|-------|------------|----|-----|
|                |       | I          | II | III |
| P <sub>1</sub> | start | 16         | 25 | 8   |
|                | end   | 14         | 24 | 11  |
| P <sub>2</sub> | start | 26         | 40 | 33  |
|                | end   | 24         | 29 | 17  |
| P <sub>3</sub> | start | 20         | 35 | 29  |
|                | end   | 23         | 22 | 19  |
| P <sub>4</sub> | start | 18         | 33 | 18  |
|                | end   | 23         | 21 | 16  |

inter-day variation between 8 a.m. and 4 p.m. in terms of reproducibility and wheal area was found, although there were significant differences in the wheal areas obtained by the different test nurses. The interpretation of this is a high constancy of the histamine reaction provided the test is performed by the same nurse. The diversity of histamine-induced wheal reaction (Table 2) is considerably less than that of allergen reaction – the maximum difference in area being 3-fold.

Our finding of a difference in wheal area between right and left arm is in accordance with that of Dirksen et al. (5). The reactions on the left arm were significantly larger than those on the right, but from a clinical point of view a difference of 4 mm<sup>2</sup> is not important, especially when the corresponding reproducibility is taken into account. The difference in reactivity of testing in the antecubital region or the wrist (9) was not found in the present study.

Although the reproducibility of the SPT is inferior to the intradermal test (8, 9, 13) the SPT histamine results show a constancy in both day-to-day variation and reproducibility if the wheal areas produced are larger than approximately 15 mm<sup>2</sup>. Therefore we suggest that histamine dihydrochloride 10 mg/ml should replace histamine dihydrochloride 1 mg/ml as positive reference in SPT\*. The advantages of SPT in terms of safety, speed and simplicity are also important in this connection.

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\* Recently, at the XV Nordic Congress in Allergology, Turku, Finland, June 1984, it was generally accepted that histamine dihydrochloride 1 mg/ml was inadequate, producing too small wheal reactions. The Nordic Standardization Committee instead recommended histamine 10 mg/ml.

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