

Estimation of skin prick test reactions with a scanning program

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We evaluated a new method of computer-based scanning of skin prick test wheal areas. To test the calibration of the program, we scanned five constructed circles of exactly defined areas between 5 and 255 mm². One of these circles was scanned in different degrees of contrast (modes), the middle mode being used in the other experiments. We also investigated the inter- and intraoperator variation. Finally, results obtained by measuring diameters of wheals and by the new method of area determination were compared for 82 histamine and 75 egg wheals. The areas found agreed well with the real areas ($P < 0.01$) (mean 97.4–100.8%), except for the smallest wheal (5 mm²) (NS). Areas obtained in the “middle position” closely resembled the real area. The intraoperator coefficient of variation (c.v.) was 1.4% (0.3–4.3), the day-to-day c.v. was 1.9% (0.2–5.3), and the interoperator c.v. was 2.3% (0.7–5.7), with a higher c.v. for small areas. Histamine and egg wheals were significantly larger with the diameter method (126% and 129%, respectively) than with the area method. The difference between the methods was most pronounced for small wheal areas. The c.v. of the scanning method was significantly lower than that of the diameter method. The new program is precise and is recommended for registration of skin test reactions in scientific trials. Although the scanner works well and has high precision, the major problem in skin testing seems still to be the reproducibility of the skin test technique employed.

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The skin prick test (SPT) is widely used in the diagnosis of immediate-type allergies (1). The most common way to evaluate the degree of IgE sensitization with SPT is either to compare the size of the allergen-induced wheal with a histamine reference wheal, or to use a fixed cutoff limit, considering a wheal more than 3 mm in diameter as a positive reaction (2). To quantitate the wheal area, usually the mean of the longest and the midpoint orthogonal diameters of the wheal is used as an estimate of skin reactivity (3).

A more reliable evaluation method would be useful to compare SPT results obtained in different centers. A precise and quantitative evaluation method would also be very useful in research projects using the SPT results for determination of the biologic activity of allergen extracts (4, 5), monitoring immunologic effects after immunotherapy (6), efficacy and kinetic studies of antihistamines (7, 8), and evaluation of the effects of

topic and systemic administration of glucocorticoids (9, 10).

Although the technique of the SPT seems simple, the reproducibility and the precision of the SPT method are often low, and vary considerably between investigators (8, 11), as shown in studies in which all areas were detected by a digitizer system by the same investigator (11).

Thus, most of the variability is attributable to the test procedure. However, this high variability is unacceptable. Therefore, all means to increase the precision should be tried. Recently, an “SPT Area Scanner” was introduced, enabling the outline of wheals to be scanned and wheal areas calculated with high precision by image-analysis algorithms (12).

The aim of the present study was to check the calibration of this instrument and to evaluate the precision and reproducibility of the SPT Area Scanner.

Material and methods

Design of the study

- 1) The calibration of the software for the scanner was tested by scanning five constructed circles of well-defined area, the calibration circles. Each circle was scanned 15 times.
- 2) In a separate experiment, one of the calibration circles used for calibration testing was scanned in three different degrees of contrast, or modes; i.e., in the "light" mode, in the "dark" mode, and in the middle of the light/dark adjustable button on the scanner. The circle was scanned five times in each mode. During the calibration as well as during all other measurements, the light/dark mode was constantly in the middle position and did not need to be adjusted.
- 3) Twenty perfectly closed figures, resembling positive SPT, with unknown area were drawn and numbered. These areas were scanned as follows:
 - a) four times by the same operator on the same day
 - b) on four different days by the same operator
 - c) by four different operators on the same day.
 Intraoperator, day-to-day-, and interoperator variability were determined by measuring the relative standard deviation (coefficient of variation [c.v.][%] = standard deviation $\times$ 100/mean of sample) between the four different measurements on each figure. To ensure lack of bias in experiment 3, the observers started scanning circle no. 1, and scanned continuously in the same sequence all circles just once. The whole procedure was repeated three times.
- 4) The areas of positive SPT of 82 patients were numbered and measured three times by the same investigator using both the scanner and the diameter method. Firstly, the quantitative differences of the area determined with the scanner and the diameter method were compared. Secondly, the reproducibility of the scanning and the diameter method was compared. Finally, the duplicate values of the SPT were compared. For both the diameter and the scanning method, the observers started scanning circle no. 1, and scanned and measured continuously in the same sequence all circles just once. The measurements were repeated twice.

Patients

Positive SPT of 82 7-month-old infants were used for estimation of wheal sizes. Subjects were tested with both histamine and egg ($n=82$) in duplicate; i.e., one on each arm. The patients were part of a study on sensitization of children at risk of developing allergy, the results of which will be presented

elsewhere. For that study, informed consent was obtained from the parents of the infants and the study was approved by the local ethics committee.

SPT

Standardized SPT were performed on both forearms of the subjects with a 1-mm SPT needle (Allergy Pricker, Dome/Hollister-Stier) (3). Histamine, 10 mg/ml (54.3 mmol/l), and frozen egg white were tested (13). The tests and the negative controls were performed in duplicate: one on each arm. The contours of the wheal were encircled after 15 min with a fine-tip pen and transferred to a record sheet by means of translucent tape. For the scanning procedure, the record sheets were photocopied. If the outline of the wheal did not form a closed figure and/or other black spots or drawings were close to the outline of the wheal, this was corrected with an ink pen and/or correction fluid (6). All SPT were performed by trained research nurses.

The SPT Area Scanner

The computer program runs on hardware compatible with an IBM AT (11) personal computer. For scanning, a common computer scanner, Handy Genius Scanner (Taiwan, KY-892030), was used. The resolution was 200 dots per inch (d.p.i.), corresponding to pixels of 0.13×0.13 mm. Each pixel was designated either black or white. The scanner should be pulled across the outline of the figure without flickering of the control lamp. After scanning, the outline was shown on the screen.

The area with or without the outline included can be calculated by the program. We always calculated the area without the outline included. If the outline of the area did not form a closed figure, this was indicated on the screen. Reports were printed and stored in ASCII text files (12).

Calibration circles

For the calibration procedure, five circles were constructed by a computer and printed by a laser printer, Hewlett Packard LaserJet (resolution 600 d.p.i.), on special, fine-structured paper with a perfect black/white contrast. Diameters were measured three times under a microscope using an electronic Vernier's calipers graded in 0.01 mm, and the mean of three determinations was used.

Determination of area by mean diameter

The area of the positive SPT was also determined by the diameter method. The mean (D_{mean}) of the

longest diameter and the midpoint orthogonal diameter was used. The diameters were measured by three different investigators, in millimeters with a transparent ruler, but calculated to half millimeters. Since diameters and areas cannot be compared for purely mathematic reasons (the c.v. differs by a factor of 2), we calculated the area of the wheal from the mean diameter according to the formula:

$$\text{Area} = \pi \times (D_{\text{mean}}/2)^2$$

Results

The proportional results, measured by the SPT Area Scanner, of the calibration circles are shown in Table 1. The median and the 95% confidence interval (CI) of each circle are also shown in this table, and they are also illustrated in Fig. 1.

One of the calibration circles (95.90 mm²) was scanned in three different modes (degrees of contrast). The differences in the proportional results of measured/defined areas are presented in Table 2.

Table 1. Calibration of SPT Area Scanner by calibration circles

Calibration circles (mm ²)	Mean (mm ²)	Area obtained by scanning (n=15)	
		Proportional result (%)	Median (95% confidence interval) (%)
5.03	4.90	97.42	97.4 (96.9–98.0)
9.51	9.44	99.26	98.8 (97.8–99.8)
50.77	50.82	100.10	100.0 (99.4–100.8)
95.9	95.52	99.60	100.0 (99.5–100.2)
255.03	257.08	100.80	100.8 (100.1–100.8)

The reproducibility of the SPT Area Scanner is shown in Fig. 2. The mean intraoperator c.v. was 1.43% (median=1.17; 95% CI 0.42–2.44%), the mean day-to-day c.v. was 1.85% (median=1.49; 95% CI 0.48–3.22%), and the mean interoperator c.v. was 2.34% (median=2.12; 95% CI –0.619–5.29%).

The areas of positive SPT with histamine, i.e., 163, and egg, i.e., 140, out of 82 duplicate tests, were measured by the diameter method, as well as by the scanning method. The histamine wheals, were all more or less perfectly closed circles. However, the configurations of the positive egg tests varied very much, and most of the areas were not circles.

The estimation ratio obtained by the diameter method of scanned areas is presented in Fig. 3 for both histamine and egg SPT, considering the SPT Area Scanner to be the most precise measurement method. The measured average percentages were, for histamine, 125.56% (range: 35.93–349.15%) and, for egg, 129.35% (range: 90.50–283.67%). In general, there was an overestimation by the diameter method of the scanned areas. However, the congruency between the two methods increased for bigger areas.

Table 2. Differences found with three different modes of contrast of SPT Area Scanner. Defined area was 95.90 mm²

Mode	Mean area (n=5) (mm ²)	Proportional results (%)
Normal	94.60	98.8
"Light"	89.51	93.3
"Dark"	97.67	101.8

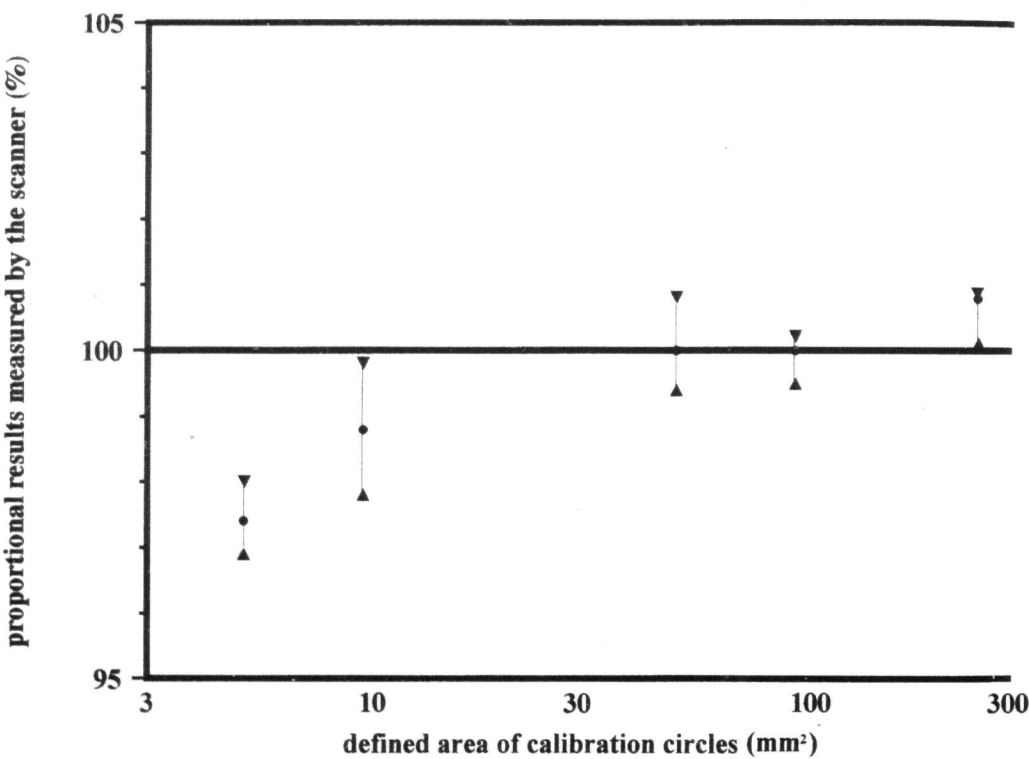


Fig. 1. Proportional results of calibration circles obtained by scanner are shown; median (n=15) and 95% confidence intervals.

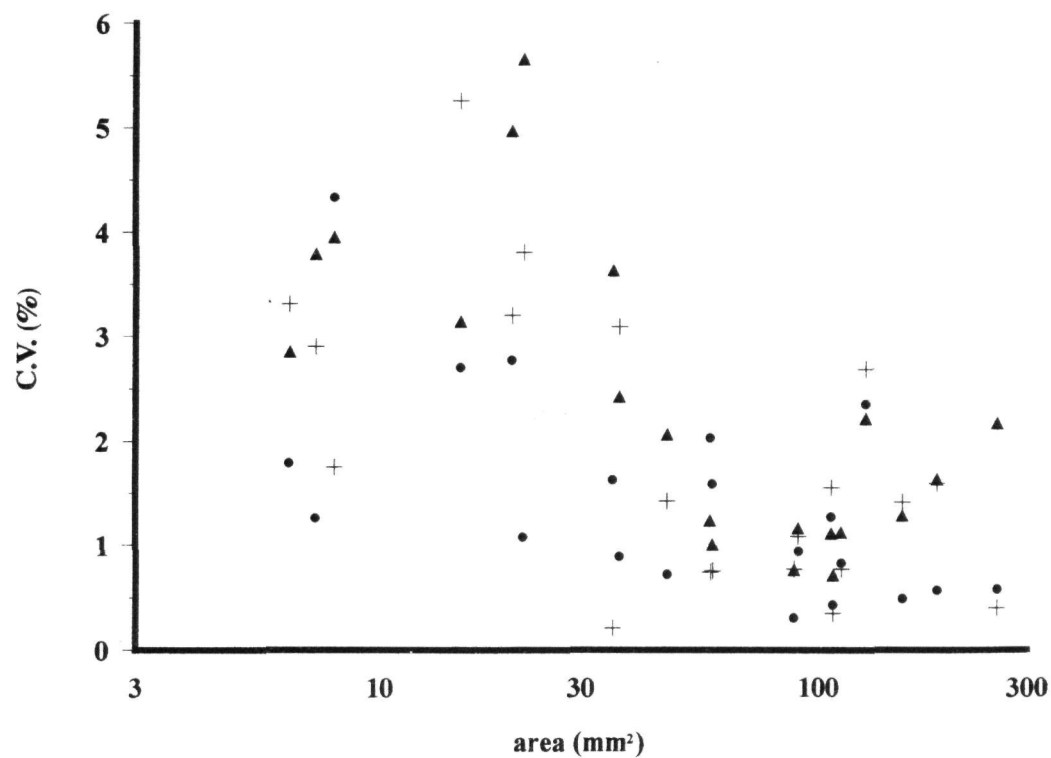


Fig. 2. Variability between four scanned samples of 20 hand-drawn circles is presented as coefficient of variation (c.v.) within same investigator on four different days by same investigator, and between four different investigators. ● = intraoperator variability, + = day-to-day variability, ▲ = interoperator variability.

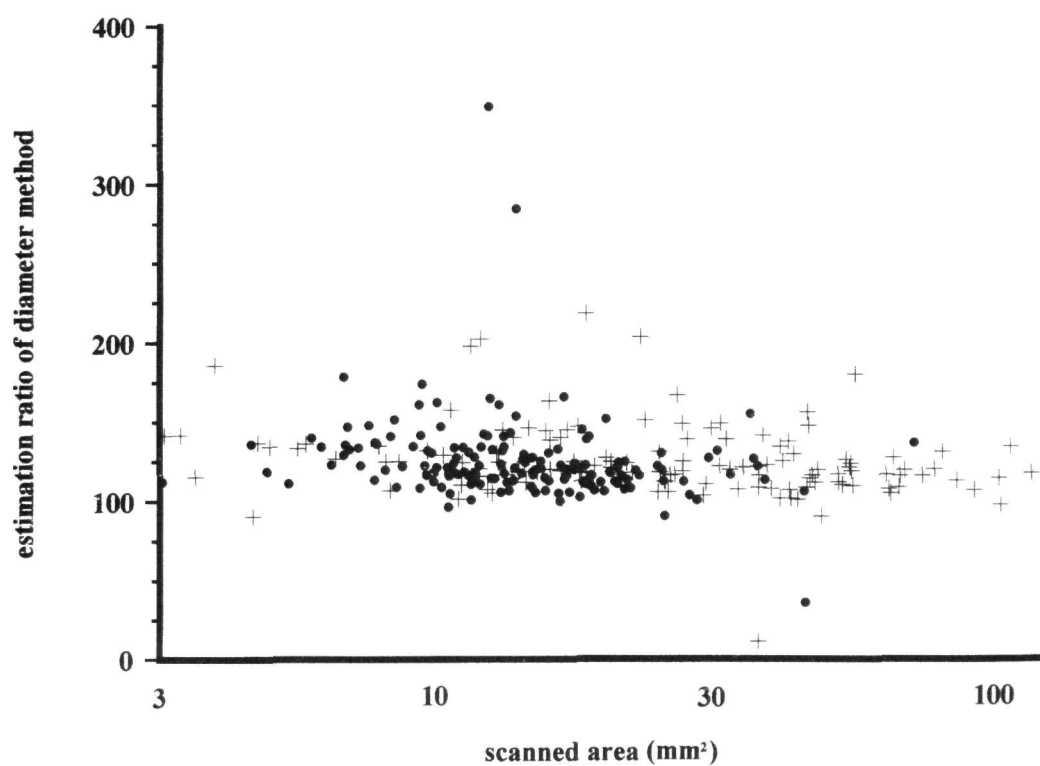


Fig. 3 Ratios of areas based on diameter method and scanning method using positive histamine (●) ($n = 163$) and egg (+) ($n = 140$) SPT are shown.

The reproducibility of the diameter method and the scanning method for positive SPT is presented in Fig. 4a and b, respectively.

Finally, the SPT were all performed in duplicate, both reactions within the same subject were compared, and their c.v. is shown in Fig. 5. The average c.v. of duplicate tests for histamine was 30.4% (range: 0.37–117.4%) and for egg 39.8% (range: 0.00–141.4%).

Discussion

In routine clinical, diagnostic work, the SPT is primarily used to screen for allergen sensitization. It is therefore usually not necessary to quantitate reactions to SPT. For research purposes (4–7, 9, 10), a more precise evaluation method would be of value. One way to increase the precision of the SPT might be to use the recently

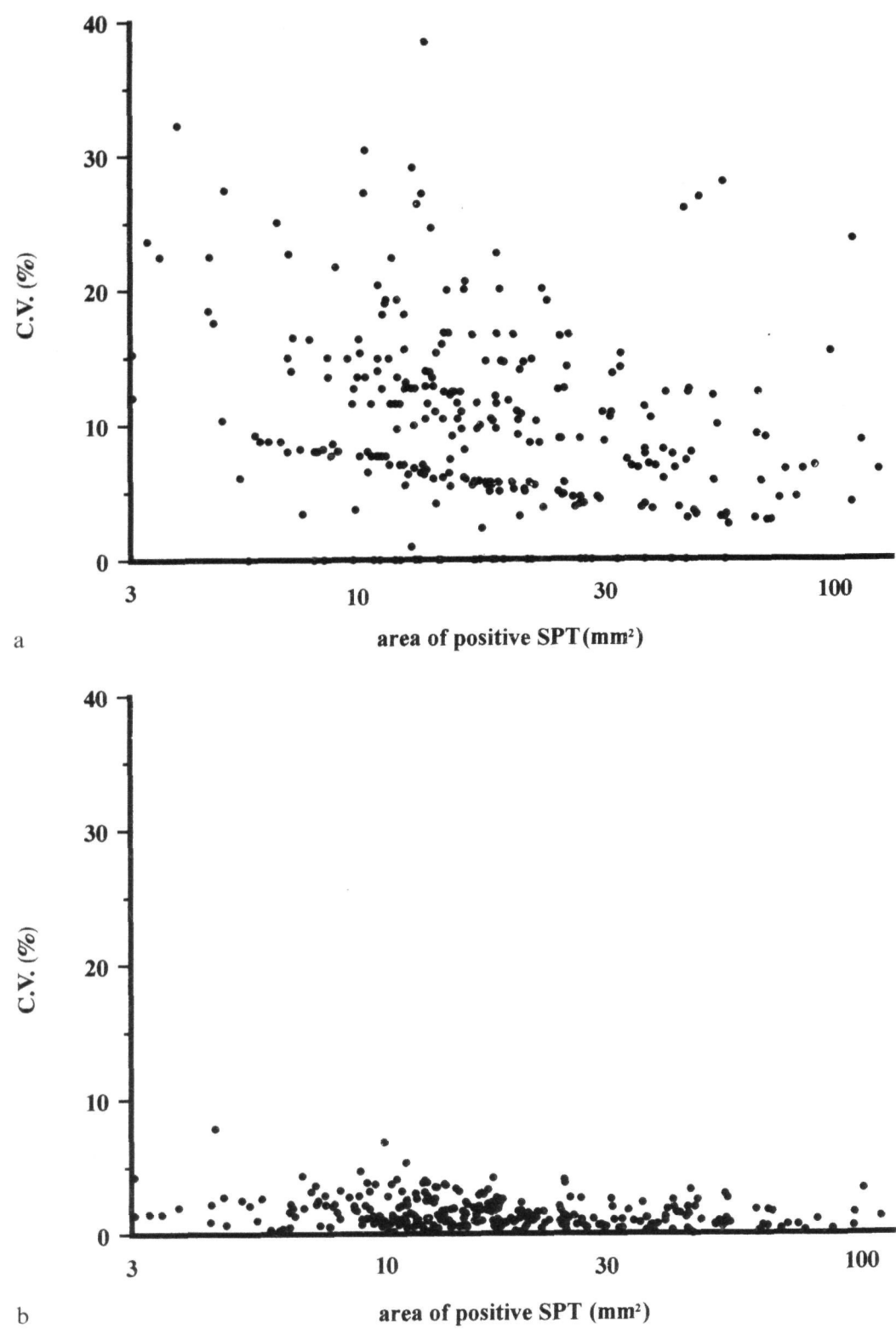


Fig. 4. a) Reproducibility expressed as coefficient of variation (c.v.) of three measurements by diameter method on each positive histamine and egg SPT ($n = 303$) is presented. b) Reproducibility expressed as c.v. of three measurements by scanning method on each positive histamine and egg SPT ($n = 303$) is presented.

developed SPT Area Scanner, which was tested in this study.

Calibration tests of the scanner proved that the proportional results were not significantly different from the defined area of the calibration circles. These results do not differ from 100% since they fall within the 95% CI. Our results agree with the results obtained in another study of the SPT Area Scanner (12).

However, concerning the calibration circles, a marginal note has to be made. Even if the circle is measured under a microscope, it is difficult to determine the exact border, resulting in a small error in the defined area of the calibration circles.

Another problem in the calibration procedure was precision in determination of the calibration circles. The scanner is accurate to 0.01 mm^2 . For sure calibration, one should have calibration circles with a known area and a precision $<0.01 \text{ mm}^2$. This is not possible in practice because, on this order of precision, temperature changes affect the validity of the measuring instrument.

With due observance of the operation procedure of the SPT Area Scanner, counting the pixels within a certain area with no contrast of the outline, one may assume that the error of the scanner is proportional to the outline of the figure. This could explain why the relative error was bigger for smaller areas.

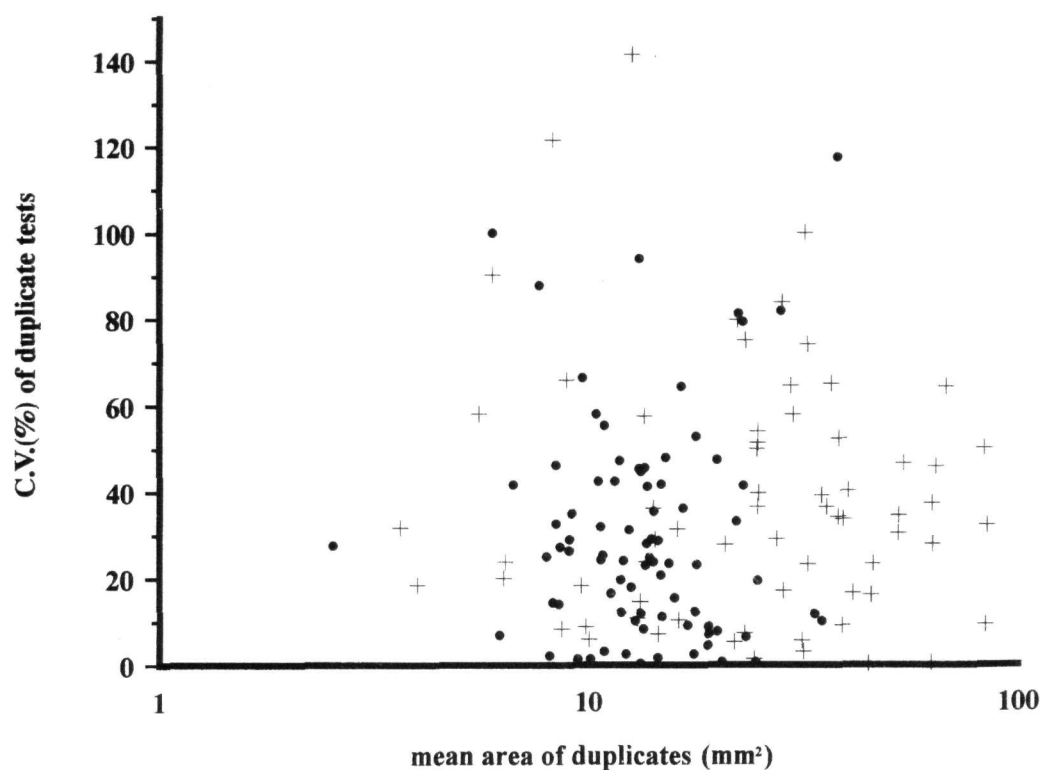


Fig. 5. Variation of duplicate SPT is expressed as coefficient of variation (c.v.) as function of mean area of duplicates. ● = histamine, + = egg.

It is important to know that configurations other than circles did not affect the validity of the scanner. In this study, analysis of the results showed that even in small areas, i.e., 5 mm², this systematic error (<1%) could be neglected in relation to the accidental error. According to the literature (10), no significant changes in reproducibility related to the geometric form of the area to be scanned were found. In this study, the reproducibility of the egg scans, which had various geometric forms, was not lower than that of the histamine scans, a confirmation of the previously described results.

It appeared from the experiment with the light/dark adjustable button on the scanner that the outcome varied 2–3% with different contrasts as indicated by this button. Although the variation was quite small, it is important to realize the effect of changing the contrast and, therefore, to keep this button in the same, middle position, especially when the scanner is used for research purposes.

Even for small areas, the reproducibility (c.v. [%]) of the scanner was below 6%, independently of by whom and when it was used. On all scans made during this study, the c.v. varied from 0.04 to 7.89. These results were even better than described by the scanner's inventors (12).

Finally, the most interesting, although not surprising, fact of the study is the reproducibility of the SPT. The reproducibility of the SPT has been investigated in a number of studies (3, 8, 11, 12, 14–16). c.v. of between 15% and >100% were described with the area method and 7% and 80% with the diameter. The most important factor in

obtaining a high reproducibility seems to be the technical performance of the test, according to the experience and skill of the tester (8, 16). In this study, the results of duplicate tests were compared by the size of scanned areas. The overall mean c.v. between the duplicates of histamine and egg was 34.75% (0.00–141.42%). This is extremely high, and thereby the precision of the area determination by the SPT Area Scanner was completely overruled by the bad technical performance of the SPT. However, a reason for this could be the fact that, for this trial, positive SPT of 7-month-old babies was used, and one can imagine that to draw accurately around their wheals is more difficult than with adults. Although such a comparison was not the main goal of the study, it could be interesting to determine these values for adults.

In conclusion, the SPT Area Scanner is a quite precise method for measuring the wheal size of positive SPT, independently of operator, geometric configuration, and wheal size, and it is thus useful. Especially when compared to the alternative method by the mean diameter, its reproducibility is high. However, the low reproducibility of the technical performance of the SPT method should be kept in mind.

References

1. Pepys J. Skin tests in diagnosis. In: Gell PCH, Coombs RRA, Lachman PJ, editors. *Clinical aspects of immunology*. 3rd ed. Oxford: Blackwell Scientific, 1975:55–80.
2. Dreborg S, editor. *Skin tests used in type I allergy testing*. Position paper. Allergy 1989; Suppl 10:44.

3. Dreborg S. The skin-prick test. Methodological studies and clinical applications. Linköping University Medical Dissertation no. 239, Linköping, 1987.
4. Nordic Council on Medicine. Registration of allergic preparations, common Nordic guidelines. NLN Publication no. 23, Uppsala, 1989.
5. Turkeltaub PC. Biological standardisation of allergenic extracts. *Allergol Immunopathol (Madr)* 1989;17:53-65.
6. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. *J Allergy Clin Immunol* 1988;82:439-46.
7. Simons FER, McMillan JL, Simons KJ. A double-blind, single dose, crossover comparison of ceterizine, terfenadine, loratadine, astemizol and chlorpheniramine versus placebo: suppressive effects on histamine-induced wheals and flares during 24 hours in normal subjects. *J Allergy Clin Immunol* 1990;86:540-7.
8. Petersen LJ, Bindslev-Jensen C, Poulsen LK, Malling H-J. Time of onset of action of acrivastine in the skin of pollen-allergic subjects. A double-blind, randomized, placebo-controlled comparative study. *Allergy* 1994;49:27-30.
9. Olson R, Karpink MH, Shelanski S, Atkins PC, Zweiman B. Skin reactivity to codeine and histamine during prolonged corticosteroid therapy. *J Allergy Clin Immunol* 1990;86:153-9.
10. Pipkorn U, Hammarlund A, Enerbäck L. Prolonged treatment with topical glucocorticosteroids results in an inhibition of the allergen-induced wheal-and-flare response and a reduction in skin mast cell numbers and histamine content. *Clin Exp Allergy* 1989;19:19-25.
11. Dreborg S, Basomba A, Belin L, et al. Biological equilibration of allergens. Methodological aspects and reproducibility. *Clin Allergy* 1987;17:537-50.
12. Poulsen LK, Lüsberg C, Bindslev-Jensen C, Malling H-J. Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993;23:61-8.
13. Dreborg S, Holgersson M, Nilsson G, Zetterström O. Dose response relationship of allergen, histamine, and histamine releasers in skin prick test and the precision of the skin prick test method. *Allergy* 1987;42:117-25.
14. Eichler I, Götz M, Jarisch R, Eichler HG, Moss R. Reproducibility of skin testing with allergen extracts from different manufacturers. *Allergy* 1988;43:458-63.
15. Vohlonen I, Terho EO, Koivikko A, Vanto T, Holmén A, Heinonen OP. Reproducibility of the skin prick test. *Allergy* 1989;44:525-31.
16. Malling H-J, Andersen CE, Boas M-B, Holgersen F, Munch EP, Weeke B. The allergy prickler. Qualitative aspects of skin prick testing using a precision needle. *Allergy* 1982;37:563-7.

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