

Objective evaluation of skin prick test reactions using digital photography

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Background: The skin prick test has been used worldwide to determine IgE-mediated hypersensitivity. However, the most current method to record this reaction has problems with accuracy and precision.

Objective: To demonstrate a new approach to measure the skin prick reaction and its kinetics with precision.

Methods: The skin prick test was induced using histamine or *Dermatophagoides pteronyssinus* antigen in 80 volunteers aged 4 and 67 years who had different skin colors. Digital photographs were obtained at 0, 3, 5, 10, 15 and 20 min. The mean wheal and erythema area as well as the erythema intensity were determined using Adobe Photoshop[®] software. The accuracy and precision of this approach were also evaluated.

Results: The digital photographic analysis measured the wheal and erythema sizes independent of the anti-

gen or skin color with precision. In addition, a new variable of this test, the skin erythema intensity, could be determined objectively using the chromaticity of reflected light.

Conclusions: Digital photographic analysis is a precise and objective method to evaluate the skin prick test reaction, which can be used independent of the patient's skin color in clinical or research settings.

Key words: skin prick test – color – computer-assisted – image processing – digital photography

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THE SKIN prick test can identify patients who have immediate hypersensitivity to a determined antigen based on the presence of specific IgE antibody on mast cells and histamine release. It is usually performed on the volar surface of the forearm after allergen and/or histamine challenge. Moreover, the technical aspects of this test are simple and can be easily learned by health workers (1).

In the majority of clinical settings, the wheal and erythema sizes are measured with a transparent ruler to determine the longest diameter and the average of the mid-point orthogonal diameter (2). Another system of determination of those parameters is based on a '+' signal when the antigen wheal or erythema is compared with that obtained with histamine (3). Unfortunately, these measurement methods have several limitations. Among these are their reproducibility, the difficulty in determining the size of irregular wheals and the fact that results are often based on wheal size only. The latter may be because of

the difficulty in analyzing erythema size or its intensity on dark skin. Moreover, there is some subjectivity in all of these procedures.

Therefore, a more accurate method for assessing the skin prick test is needed for both clinical as well as research settings. This new technique should be simple, objective, quantitative and reproducible so as to measure accurately allergen sensitivity and the success of immunotherapy or pharmacotherapy (4, 5). The procedure should also be easily transferable to medical/research centers worldwide.

The aims of this study were to describe a new objective method for measuring the skin prick test with all skin phototypes, and to determine its accuracy and reproducibility.

Methods

Study design

This research was performed as an experimental and descriptive study of the skin prick test

kinetics based on digital photograph. The study protocol was approved by the local Research Ethics Committee, and all volunteers or parents gave their signed informed consent. This report was designed to determine the accuracy and reproducibility of the digital photographic system when compared with other evaluation procedures for a wheal and erythema response.

Subjects

Eighty volunteers, 31 men and 49 women, aged 4–67 years, were randomly selected among patients and staff members over an 8-month period in the University Hospital, Curitiba, Brazil. The subjects had not taken antihistamines, mast cell stabilizers, corticosteroids or other drugs that might interfere with the procedure in the last 10 days. None of them were in an immunotherapy program or had dermatological disease in the tested area. All of them had their phototype determined by the Fitzpatrick classification system before the tests were performed (6).

Skin prick tests

For all skin prick tests, a drop of histamine (10 mg/mL, IPI-ASAC, São Paulo, Brazil), *Dermaphagoides pteronyssinus* extract (112,900 UBE/mL, IPI-ASAC) or saline solution (IPI-ASAC) was applied at different points on the anterior portion of the left forearm. The skin was then punctured using a 26-gauge needle.

A self-adhesive white circle of a known size was placed next to the test area for dimension reference. Markers with red, green, blue and black colors were positioned nearby for color reference.

Digital photography

An Olympus 650C LCD camera was held fixed at 400 mm from the subject's forearm. The pictures of the test areas contained 1280×1024 effective picture elements (pixels) of resolution, and were taken in a closed room, with illumination provided by two photoflood lamps (BCA no 1 115–120 V – General Electric Co., Schenectady, NY, USA) positioned on each side of the subject's forearm. Photographs were obtained at 0, 3, 5, 10, 15 and 20 min after the test was initiated, and these were transferred to a computer for analysis using Adobe Photoshop® 6.0 software.

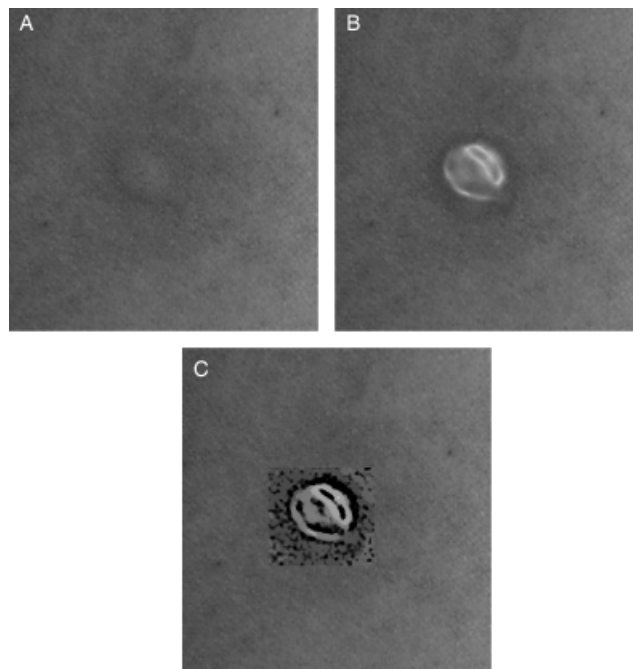


Fig. 1. Wheal size delimitation by digital photography analysis. (A) Wheal induced by histamine at 10 min. (B) After the first filter from Adobe Photoshop®. (C) After the second filter from Adobe Photoshop®.

Wheal area by digital photography

The wheal area was selected and ascertained using two Adobe Photoshop® filters: a plastic wrap and poster edges (Fig. 1). The number of pixels inside the selected area and the reference circle were determined. The wheal area in mm² was established by a simple mathematical correlation between the two areas. The results were expressed as mean $\pm$ standard error.

Erythema area by digital photography

The region of the erythema reaction was selected. The colors and luminosity levels of this area were adjusted to accent the reaction (Fig. 2). The erythematous area was selected and the pixels were quantified. The area in mm² was assessed as described above for wheal size, and the results were expressed as mean $\pm$ standard error.

Erythema intensity by digital photography

The Adobe Photoshop® software can also measure the chromaticity and the luminosity of reflected light. Therefore, we measured the chromaticity and intensity of light reflected from the skin in the tested area compared with an adjacent untested area. The erythema intensity analysis was based on the RGB (Red, Green, and Blue) model. Briefly, the red, blue and green

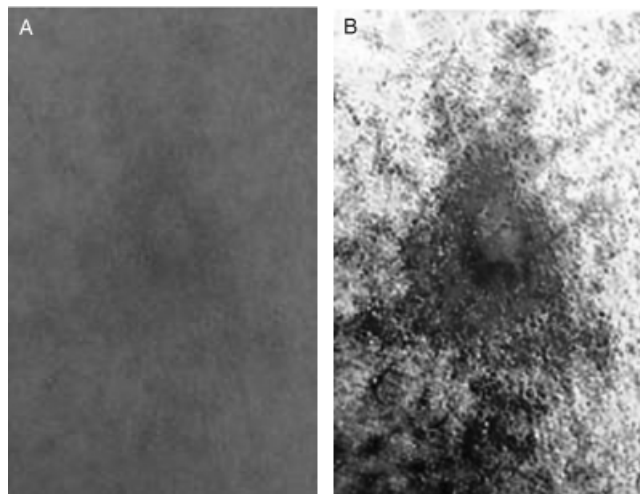


Fig. 2. Erythema digital enhancement by digital image transformation for size determination. (A) An erythema induced by histamine at 10 min. (B) Erythema after color adjustments using Adobe Photoshop®.

colors were converted into an intensity scale ranging from 0 to 255. The mean brightness values of the RGB channels were obtained for the tested area compared with an adjacent non-tested area of the same size. For each determined parameter, control values were subtracted from the histogram median of the tested area. The color intensity differences were expressed as the mean $\pm$ standard error.

Accuracy and precision tests

The digital photography method was compared with other reading systems. For one comparison, a transparent ruler was used to measure the longest and the mid-point orthogonal diameter. The mean diameter ($(D_1 + D_2)/2$) was calculated. For another comparison, known as planimetry, a transparent adhesive tape was placed on the patient's arm and the borders of the wheal and flare were drawn. The wheal and erythema drawing was placed on a white record sheet with a circle of a known area. The drawing was digitized using an image scanner and the drawing areas were determined using the methods described above to measure the wheal area by digital photography. These methods were used at the time of digital photography and at the same time points, and were expressed as mean $\pm$ standard error.

We also evaluated within-observer and between-observer reproducibility. To accomplish this task, 36 digital pictures were analyzed by a single observer or by seven observers on two

separate occasions. All observers were blinded regarding the picture identification and measurement results throughout the analysis.

Statistics

All results were expressed in graphs as the mean standard error of the mean (SEM). We used the χ^2 -test to examine the distribution of a categorical response variable as conditioned by the values of another categorical variable. The kinetics of the response were compared by ANOVA for repeated measurements. We compared means using Student's *t*-tests. To compare the different methods, correlation was applied. ROC curve and κ were also used to study the accuracy of this new method. The results of intra- and inter-observers were determined by the coefficient of variation (CV). All statistical analyses were performed using the JMP 5.0 (SAS Institute, USA) statistical program. A value of $P < 0.05$ was considered statistically significant for all analyses.

Results

Population characteristics

The skin prick test was performed on eighty volunteers, 49 (61.2%) of whom were females. The mean age was 25.2 years $\pm$ 13.4. Thirty-five of them presented with a positive skin reaction for *D. pteronyssinus*. Fifty-eight of 80 volunteers were classified as phototype II or III and 22 of them as phototype IV or V. Considering the fact that the distribution of positive results among the different skin phototypes might interfere with the analysis, we performed a χ^2 statistical analysis to determine whether they were independent variables. This analysis did not demonstrate a difference between the *D. pteronyssinus* and histamine results and skin phototype, which means that the test positivity and skin phototype were independent variables.

Wheal and erythema areas

The development of the mean wheal area induced by histamine and *D. pteronyssinus* was obtained at each timepoint, which revealed a progressive increase in this parameter over time. The mean peak size occurred at 20 min ($37.11 \pm 4.0 \text{ mm}^2$) when induced by histamine and at 20 min by *D. pteronyssinus* ($39.61 \pm 10.17 \text{ mm}^2$). There was no statistically significant difference between their kinetics ($P = 0.81$) (Fig. 3).

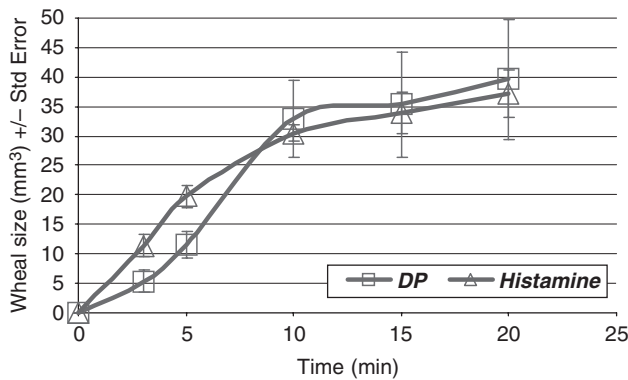


Fig. 3. Wheal kinetic by digital photography analysis. The lines indicate the wheal area of the histamine (triangle), and *Dermatophagoides pteronyssinus* (square) determined by digital photography analysis. The area (mm²) was evaluated at several points until 20 min after antigen or histamine challenge. Results are reported as mean $\pm$ SEM of all volunteers.

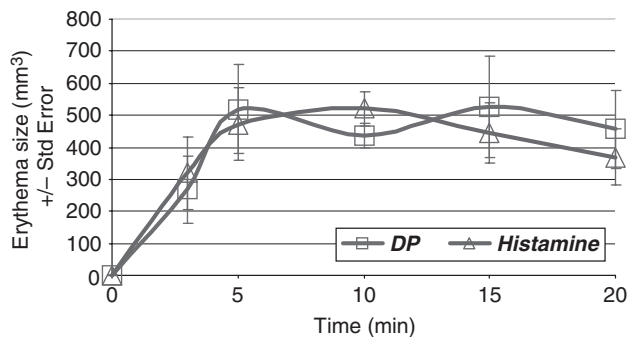


Fig. 4. Erythema size kinetic by digital photography analysis. The erythema area was determined after digital picture color adjustment by Adobe Photoshop[®] over time. The results are reported as the mean in mm² $\pm$ S.E.M. The histamine (triangle) erythema was compared with *Dermatophagoides pteronyssinus*.

The erythema size reached a plateau at 5 min and maintained this plateau (500 mm²) through 15 min. These results were observed in patients of all phototypes. There was no difference between erythema kinetics induced by histamine or *D. pteronyssinus* extract ($P = 0.60$) (Fig. 4).

Erythema intensity

The kinetics of the development of erythema intensity in response to histamine or *D. pteronyssinus* challenge was similar in all patients, independent of skin phototype. Therefore, the data were grouped, and the mean for all points was determined and analyzed. The red color varied significantly during the time of observation when compared with normal skin. The red intensity at 0, 3, 5, 10, 15 and 20 min was 0, 4.25 ± 0.96 , 5.10 ± 1.04 , 3.75 ± 1.80 , 7.35 ± 0.85 and 8.05 ± 0.69 , respectively. The intensity of this color was

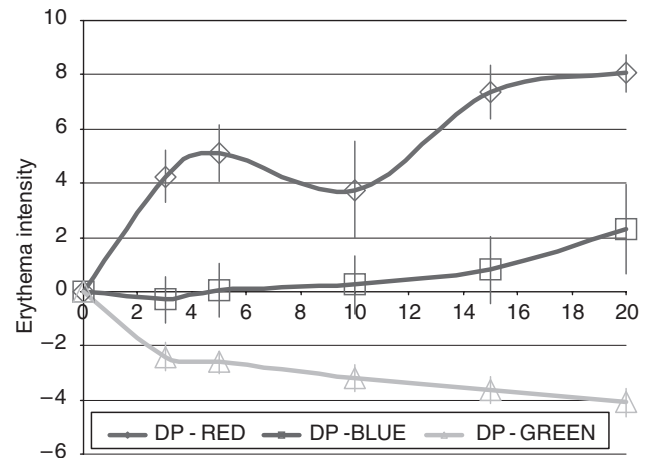


Fig. 5. Erythema intensity kinetic induced by histamine by digital photography analysis. The red, blue and green color intensities from normal and histamine challenged skin were determined by Adobe Photoshop[®] over time. The results are reported as the mean difference between the areas for each basic color $\pm$ SEM.

statistically higher in the prick test area at 5, 10, 15 and 20 min than in the control area ($P < 0.001$). The green color intensity was reduced at 3, 5, 10, 15 and 20 min ($P < 0.001$). The blue intensity was the only basic color that did not change with time ($P = 0.67$) (Fig. 5). There was no statistically significant difference between *D. pteronyssinus* and histamine erythema intensity ($P = 0.723$).

Accuracy test

When the three approaches (digital photography, ruler and adhesive tape methods) were compared, they gave similar results regarding wheal size. Moreover, the degree of agreement among the three methods was greater when the wheal size was larger. The kinetics for wheal size development was also similar among the tested methods. Using digital photography, wheal size reached a plateau at 20 min after challenge and this plateau was reached with the other methods by 15 min post-challenge. There was a correlation between the digital photography, ruler and tape adhesive methods at 10 min post-challenge ($P < 0.0001$; r^2 adjust = 0.54; r^2 adjust = 0.71, respectively).

When this same analysis was applied to the development of erythema, there was only a weak association among the different methods.

The ruler method is the most widely used and accepted technique for measuring skin prick test results and is considered positive if the mean diameter of the wheal is 3 mm or greater. An ROC analysis revealed that a wheal size of 12.48 mm² was considered positive for skin prick reactions

analyzed by digital photography ($P = 0.05$; $1 - \alpha = 0.95$), which was higher than the one based on circle area ($\pi \times r^2$), which was estimated as 7.08 mm^2 . The value of κ for these numbers was 0.82 ± 0.03 (SE). Given an area of 12.48 mm^2 , the sensitivity of a positive prick test detected by digital photography was 87.2% and 100% specific.

Reproducibility test

To determine the reproducibility of the wheal and erythema sizes determined by digital photography, precision profiles based on the CV for 36 pictures at different times and from different phototypes were determined. The intraobserver variability was established by two measurements from the same operator one week apart. Seven different operators determined the interobserver variability on two different occasions.

The mean interobserver CV was 5.95% (median = 1.87% CI 4.58–9.13) for wheal size. The mean intraobserver CV was 0.53% (median = 0.15% CI 0.71–1.39) for the same parameter. For erythema size, the mean interobserver CV was 7.58% (median = 3.52% CI 5.95–11.72), and the mean intraobserver CV was 3.69% (median = 3.99% CI 0.31–7.69).

Discussion

No standardized system exists for recording and interpreting skin prick tests, although various methods and devices have been developed. Moreover, most of these methods for skin prick test evaluation are not practical for use in a busy clinical practice (1). In this study, we present an objective, precise and practical method for skin prick test analysis by digital photography, which can be used in any modern office or laboratory setting for patients with any skin color.

The majority of the methods for skin prick test analysis are based on the wheal size after antigen extract challenge. The most common procedure for recording the reaction is the ruler method (2). However, its precision varies considerably between centers, and this variability may be because the wheal reaction is frequently irregular in shape and thus the diameter of the reaction is not an accurate reflection of the size of the response. As a result, for example, in most centers involved in trials in Europe, the coefficient of variation was between 15% and 30% using the wheal size

diameter (7, 8). Other methods have been examined in an attempt to increase the reproducibility and precision of skin prick test evaluation, but these methods are frequently too technically demanding to be used in routine practice (9–14). Therefore, a simple and precise method for skin prick test evaluation is still needed. Such a method may allow different research centers and physicians to compare their results.

To this end, we developed a method to evaluate skin prick tests by digital photography. In our study, the wheal areas were determined by digital image analysis, which eliminates the variability that is inherent in any method that requires a human component in the data-collection phase. We investigated the precision of this method by inter- and intraobserver variation, and the accuracy by comparing this new method with two other methods. By digital photography, the intraobserver and interobserver CV for wheal areas were 0.53% and 5.95%, respectively. The digital photography method was equivalent or superior to the other two methods and was clearly the most objective of the three approaches. Moreover, while the most widely accepted threshold for a positive wheal reaction measured by conventional techniques is 3 mm^2 or greater, the increased sensitivity of the digital photography method had an enhanced threshold of 12.49 mm^2 (2).

While the erythema associated with the skin prick reaction has been considered as another parameter of the response that could be measured, it has not been extensively used because of the difficulty of assessing it in dark-skinned individuals. However, using sensitive color digital cameras, it is possible to measure this erythema response accurately, even in dark-skinned people. Therefore, it is perhaps not too surprising that the results obtained using digital photography were different from those obtained using the ruler or adhesive tape method, probably because of the difficulty and subjectivity in measuring erythema by eye, especially on dark-skinned individuals.

Erythema intensity has never been assessed before in skin prick reactions and represents a new inflammatory parameter of the reaction. By measuring the combination of the RGB intensities, we were able to observe the development of the flare reaction intensity over time. Our findings are consistent with those of Setaro and Sparavigna (15) for other dermatological conditions. They found a significant reduction in the

green color channel and an increase in red color values. Moreover, Boysen et al. (16) evaluated skin erythema by digital photography in dogs. Comparable with our data, these authors were able to differentiate various degrees of erythema by digital photography that was not possible by visual observation or chromametry.

One possible limitation of this digital photographic system is the need for special lighting sources. However, simpler lighting sources may also be sufficient, especially when this is combined with the use of different filters and other processing techniques. Moreover, the digital photographs used in this study were of 1280×1024 pixels. It is possible that higher resolution photographs may obviate any limitation of this digital photographic approach.

Finally, this process has other advantages. Digital photography produces legal documentation of the skin reaction and allows access to these records at a variety of different locations. It also allows for the seamless integration of all patient images (e.g. radiographic, pathologic) into the medical record. The images can be either printed or stored in image databases.

In summary, we demonstrate an objective, precise and simple method for analyzing skin prick tests by digital photography. This method is appropriate for routine use or for research trials, which require high-quality and consistent results. In addition, this method allows us to determine the kinetics of the wheal and erythema size even in volunteers with a type IV or V skin phototype. Moreover, a new parameter, erythema intensity, can be studied. Compared with other methods for assessing the skin prick reaction, this procedure is more objective and is able to capture all manifestations of the skin prick reaction simultaneously.

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