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Skin Scrape Test: An Inexpensive and Painless Skin Test For Recognition Of Immediate Hypersensitivity In Children And Adults

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Abstract: *Background:* Skin testing is a mainstay in allergology, and its importance is increasing in several fields. The ability to choose the most suitable technique according to the clinical setting is an advantage for the medical team.

Objectives: To describe in detail an alternative technique of the coetaneous allergy test (skin scrape test) conceived as a variation of the former skin scratch test; to evaluate its value as a tool for diagnosis of immune sensitization; and to compare its accuracy with the skin prick test.

Methods: The skin scrape test and skin prick test were performed side by side with the same allergen extracts in 162 human subjects classified in two groups according to the known presence or absence of serum specific-IgE to these allergens.

Results: The sensitivity of the skin scrape test to detect immediate reactions was 85.0%. The sensitivity of the skin prick test was 86.5%. The sensitivity of both techniques analyzed together as a unique procedure was 94.2%. The specificity of the skin scrape test was 90.1%. The specificity of the skin prick test was 72.9%. The specificity of both tests analyzed together as a unique procedure was 70.5%.

Conclusions: The skin scrape test is an alternative and complementary technique for allergic skin testing, and it is able to detect IgE-specific immune sensitization without the disadvantages of the skin scratch test. The skin scrape test has similar outcomes to the skin prick test.

Keywords: Immediate Hypersensitivity (Medline ID D006969), Diagnosis (Medline ID D003933), Skin Test (Medline ID D012882), Dermatophagoides Antigens (Medline ID D039741), Child (Medline ID D002648), Atopic Dermatitis (Medline ID D003876), Rhinitis (Medline ID D012220), Asthma (Medline ID D001249).

INTRODUCTION

The skin scratch test was the first test described as a tool for allergy diagnosis. It was reported in 1873 by Charles Blackley, who abraded a quarter-inch area of his own forearm to correlate his nasal symptoms with *Lolium italicum* pollen grains [1]. After Blackley's initiative, skin scratch tests were used during a long period until the appearance of the skin prick test(SPT) in the early 1950s [2], which, despite the use of different devices [3], was easier to submit to a standardization process [4]. The SPT is currently a wellestablished technique for the *in vivo* diagnosis of IgEmediated sensitization, but it is not always a reproducible technique due to numerous interfering factors [5]. The major inconvenience of the former skin scratch test is the associated skin trauma leading to false-positive results depending upon the type of device used and the strength applied by the health care professional to scarify the skin [6]. One of most commonly used devices to perform the skin scratch test is the blood lancet [7]. Blood lancets were not designed to scarify skin and were used upside-down to scratch the skin with their blunt base. Usually, the bottoms of the lancets are not adequately polished to provide a burr-free edge, which is what accounts for the production of actual trauma to the skin. In the chamber-scarification technique, the authors used the point of a needle to break the skin in a crisscross pattern before applying the allergen [8]. The literature suggests that the skin scratch test has been abandoned due to poor reproducibility, great discomfort and the possibility of residual pigmented or depigmented areas for some time afterwards [9, 10]. Here, we propose an alternative method for performing skin testing using a modified skin scratch test technique called the skin scrape test (SST), which was developed to avoid the original inconveniences of the early skin scratch test and the current SPT. We describe this method in detail with the objective of providing a standard methodology both for daily clinical work and for use in multi-center studies in order to effectively determine the predictive value of skin tests [11]. To evaluate the accuracy

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of the SST, we compared it with the SPT. We performed the tests side by side and employed standardized allergens that patients had previously been diagnosed, by the quantification of specific IgE (s-IgE), as being sensitized to or not sensitized to. This accuracy study, which was performed as recommended by the GRADE approach [12] (Grades of Recommendation, Assessment, Development and Evaluation), allowed us to determine the sensitivity and specificity of the SST in this specific selected population using the s-IgE as a proxy of sensitization [13-15]. Patients were analyzed according to the presence or absence of medically diagnosed atopic diseases such as persistent rhinitis, asthma and/or atopic dermatitis. These conditions were chosen due to their particular association with IgE-mediated immune reactivity [16].

METHODS

Study Design and Subjects

The study was approved by the ethical review board of the University of Sorocaba registered at the Plataforma Brasil (CAAE 07453312.2.0000.5500), and it was conducted according to the principles of the Declaration of Helsinki. We examined 74 male and 88 female subjects (1 to 68 years, mean 26.1 +/- 18.3 years). To evaluate the utility of the SST, we compared it with the SPT performed at the same time with the same allergen solution in patients previously diagnosed by serum s-IgE (CAP Systems Pharmica; results expressed as kU/L) as being sensitive or not to these allergens [17]. A group of atopic patients was used to study the sensitivity of the skin tests. This group was composed of 125 patients with s-IgE> 1.0 kU/L to at least one of three house dust mite (HDM) antigens (Dermatophagoides pteronyssinus, Dermatophagoides farinae and Blomia tropicalis). Some patients had increased s-IgE to more than one mite and were included in two or three analyses, totaling 208 pairs of tests. The control group consisted of asymptomatic subjects to study the specificity of the skin tests and was composed of 37 subjects with s-IgE< 0.35 kU/mL to at least one of the following five allergens: the three HDM mentioned above plus Aspergillus fumigatus and Hevea brasiliensis. In this group, 122 pairs of tests were performed.

Skin Testing Methodology

Glycerinated saline diluent was used as a negative control. Histamine sulfate 1 mg/mL was used as a positive control. The SPT and the SST were performed by trained and supervised health care professionals on the volar aspect of the forearm in adults and children and on the back of toddlers and babies after withholding antihistamines for at least 10 days. Babies were tested on a parent's lap. Toddlers were tested while they were seated over the table edge in front of their mother. The SPT was performed with a disposable sterile acrylic pricker with a 1-mm lancet over a blunt basis (Punctor[®] purchased from Alko do Brasil). The pricker was introduced at a 90° angle perpendicular to the skin, through allergen solution, retrieved after 5 seconds and then discarded. To scrape the skin, we used the bevel of a sterilized and disposable 18-G $1\frac{1}{2}$ (1.20 x 40) hypodermic needle (standard bevel). The skin was not scraped with the needle's point. Its hub (allowing some flexibility in touching the skin) gently held the needle at a 10° to 25° angle. The health care professional scraped the skin with the help of the bevel's edge in only one direction. The bevel did not scrape the skin backwards, and its movement resembled a broom cleaning the floor. The objective was to wipe the most superficial epidermal layer instead of traumatizing or scarifying the skin. This movement was repeated approximately3 to 6 times (depending on the skin thickness), until the health care professional observed a thin homogeneous desquamation or a slight hyperemia (see Video 1). If scraping occurred beyond this depth, a skin scratch test would be performed instead of an SST. The extension of the scraped area was irrelevant because the tested area depends on the diameter of the drop applied. The width of the scraped area measured at least 3 mm wide. The health care professional scraped all of the areas to be tested in a single step (a single needle was used to perform all of the tests). During the first scrape, the health care professional counted the number of movements necessary to produce desquamation and repeated the same procedure on other are as to perform a homogeneous test. Sometimes, the scraped area was hardly visible, and it was helpful to mark the areas with a dermographic pen prior to scraping. Good illumination in the procedure room was essential for the skin tests. The allergens solutions are applied after scraping. Optionally, after scraping and before applying the allergens, the health care professional can wait 15 minutes to observe an occasional dermographism that could invalidate the test. Disrupting the stratum corneum barrier by the SST makes the skin permeable to soluble allergens; however, they do not gain immediate access to reactive layers. Thus, unlike the SPT (in which allergen solution is directly introduced via a 1-mm lancet into the skin, thus allowing the immediate cleaning of the allergen solution), when performing the SST, the allergen solution remained on the skin to allow absorption until the final reading was made at 15 minutes. At this time, the wheal's longest diameter (WLD) was assessed. We perform a negative control to draw the cut-offs for positive and negative tests. A wheal reaction was defined as positive if the WLD was $\geq 3 \text{ mm } 15$ minutes after the application of the allergen extracts and after the subtraction of each patient's reaction to the negative control [10, 18].

The SST is a more convenient technique for use in small children because it is painless and promotes fewer struggles than the SPT. Children usually struggle more during pricking than during scraping sessions. Most children do not struggle during dermographic marking or allergen application. Struggles are less inconvenient during scraping because allergens have not yet been applied. One can apply allergens during a calmer second step some minutes after scraping. Struggles during pricking (when the allergens are already on the skin) make the procedure more difficult due to the increased possibility of dislodgements of solutions and the mixing of allergens. In this case, it is more convenient to extend the steps, apply one allergen, prick and dry the solution immediately after the prick before the next allergen application. The possibility of recognizing dermographism before applying the allergen solution is a clear advantage of the SST over the SPT because in the SPT, the trauma associated with puncture may produce an immediate reaction that sometimes may be indistinguishable from the reaction produced by the allergen.



Fig. (1). Dropping of antigen extract solution onto the plastic antigen dispenser being applied to the scraped area. On the table are the container to keep the unused disposable dispensers (without cover) and the container to dispose the used dispensers (with a hole in the cover).

Disposable Allergen Dispenser

We used a disposable allergen dispenser to intermediate the allergen solution between the vial and the scraped skin. The allergen dispenser is a plastic spatula with an enlarged end where the allergen solution was dropped before being applied to the scraped area. Immediately after allergen application, this plastic dispenser was discarded. This procedure was also adopted for the performance of the SPT in order to standardize allergen application. The disposable allergen dispenser allows better visual control of the drop's size and avoids the waste of allergen solutions. To discard the dispenser; we used a steel container topped with a cover with a narrow hole to allow the health care professional to dispose of the dispenser but not to (unadvisedly) retrieve it. Another container (without a cover) holding unused dispensers was also available on the examination table (See Fig. 1).

Statistical Analyses

Group comparisons of the skin test outcomes were performed with paired and unpaired t tests. The data are reported as the arithmetic mean with 95% confidence intervals (CI). Box column graphs with 95% CI whiskers were plotted between the means of the SPT WLD and the SST WLD. Paired correlation charts between the SPT WLD and the SST WLD were plotted, and the Spearman rank correlation was used to analyze the results.

Prospective contingency tables analyzed by Fisher's exact tests were used to compare the categorical diagnostic performance between tests [19]. The sensitivity of the skin tests was calculated by grouping WLD results of \geq 3mm into a single categorical value. The specificity of the skin tests was calculated by grouping WLD results of <3 mm in to a single categorical value. For all analyses, a p-value of less than 0.05 was considered statistically significant.

Statistical analyses were performed with GraphPad Prism for Windows (version 5.0; GraphPad Software, Inc., San Diego, CA, USA).

Schematic of the method of skin scrape test

SCHEMATIC PRESENTATION OF THE METHOD OF SKIN SCRAPE TEST

- 1. Be sure the patient is withholding antihistamines for at least 10 days.
- 2. Disinfection of the area (volar aspect of the forearm in adults/children and on the back of tod-dlers/babies).
- 3. Mark the area with demographic pen.
- 4. Scrape the area with the bevel of a 18-G $1\frac{1}{2}$ (1.20 x 40) hypodermic needle (standard bevel) until the appearance of a thin desquamation or a slight hyperemia.
- 5. Optionally wait 15 minutes to observe occasional dermographism.
- 6. Apply the allergens and control solutions with help of the allergen dispenser over each scraped area.
- 7. Wait 15 minutes.
- 8. Dry the area with an absorptive paper.
- 9. Measure the wheal/erythema longest diameters.
- 10. Optionally mark the wheal with a demographic pen and transfer the mark to a graph paper with help of a transparent tape.
- 11. Wash the area.

	Mean Wheal's Longest Diameter		
	SPT	SST	
D. pteronyssinus	6.1 mm	8.2 mm	
D. farinae	6.5 mm	7.9 mm	
Blomia tropicalis	5.3 mm	4.8 mm	
Average mean	6.0 mm	7.6 mm	
Positive control	6.5 mm	8.3 mm	

Table 2. Accuracy (Number of Positive Tests) Followed by the Percentage from Total (Sensitivity) and the False Negative Proportion of the Skin Scrape Test (SST), the Skin Prick Test (SPT) and Both Simultaneous Tests in Patients with s-IgE > 1.0 kU/L to Dermatophagoides pteronyssinus (D1), Dermatophagoides farinae (D2) and Blomia Tropicalis (RD201)

	SST Accuracy (Sensitivity)	SST False Negative (Proportion)	SPT Accuracy (Sensitivity)	SPT False Negative (Proportion)	SST+SPT Accuracy (Sensitivity)	SST+SPT False Negative (Proportion)
D1	83	9	81	11	88	4
(92 tests)	(90.2%)	(9.7%)	(88.0%)	(11.6%)	(95.5%)	(4.3%)
D2	67	10	67	10	73	4
(77 tests)	(87.0%)	(12.9%)	(87.0%)	(12.9%)	(94.8%)	(5.2%)
RD201	27	12	32	7	35	4
(39 tests)	(69.2%)	(30.7%)	(82.0%)	(17.9%)	(89.4%)	(10.2%)
Total	177	31	180	28	196	12
(208 tests)	(85.0%)	(14.0%)	(86.5%)	(13.4%)	(94.2%)	(5.7%)

Table 3. The Skin Prick Test (SPT) and the Skin Scrape Test (SST) with the Corresponding Antigens According to Medical Diagnosis in Patients with s-IgE > 1.0 kU/L to Dermatophagoides Pteronyssinus, Dermatophagoides farinae and/or Blomia tropicalis

Medical Diagnosis	Mean Age (Years)	Mean s-IgE (kU/L)	Positive to the SPT (Sensitivity)	Positive to the SST (Sensitivity)	Positive to Both SST and/or SSP (Sensitivity)
Atopic dermatitis (79 tests)	19.9	47.0	68 (86.0%)	74 (93.6%)	77 (97.4%)
Asthma (55 tests)	18.6	40.3	46 (83.6%)	48 (87.2%)	51 (92.7%)
Rhinitis (171 tests)	20.3	35.1	152 (88.8%)	144 (84.2%)	162 (94.7%)

RESULTS

Information on current rhinitis, asthma and atopic dermatitis was available for all subjects. The mean SPT positive control WLD was 6.5mm (SD 2.7). The mean SST positive control WLD was 8.3mm (SD 4.6). The mean SPT and SST allergens WLD in patients with s-IgE> 1.0kU/mL to HDM are shown in Table 1. In this group, the sensitivity of the SST to detect HDM sensitization was 85.0% and the sensitivity of the SPT was 86.5% (p>0.05 Fisher's exact test - see Table 2). Some patients did not react to the SPT but reacted to the SST, and others reacted to the SPT but did not react to the SST. Considering both tests analyzed together as a unique procedure, the sensitivity increased to 94.2%. Table **3** presents the sensitivity of the SPT and the SST according to clinical diagnosis. Among the three conditions studied, a topic dermatitis showed better predictability by the SST alone (sensitivity of 93.6%) and in association with the SPT (sensitivity of 97.4%), but the differences with the other conditions were not significant using Fisher's exact test (p > 0.05). In the group of patients with s-IgE< 0.35 kU/mL, the specificity of the SST was 90.1% and the specificity of the SPT was 72.9% (p > 0.05 Fisher's exact test). The specificity

Table 4. Accuracy (Number of Non Reagent Tests) Followed by the Percentage from Total (Specificity) and False Positive Proportion
of the Skin Scrape Test (SST), the Skin Prick Test (SPT) and both Simultaneous Tests in Patients with s-IgE < 1.0 kU/L to
Dermatophagoides Pteronyssinus (D1), Dermatophagoides Farinae (D2), Blomia tropicalis (RD201), Aspergillus fumigatus
(M3) and Hevea Brasiliensis (K82)

	Non-Reactive to the SST (Specificity)	SST False Positive (Proportion)	Non-Reactive to the SPT (Specificity)	SPT False Negative (Pro- portion)	Non-reactive to SST + SPT (Specificity)	SST + SPT False Positive (Proportion)
D1	26	2	19	9	19	9
(28 tests)	(92.9%)	(7.1%)	(67.9%)	(32.1%)	(67.9%)	(32.1%)
D2	22	3	16	9	16	9
(25 tests)	(88%)	(12%)	(64%)	(36%)	(64%)	(36%)
RD201	21	2	15	8	15	8
(23 tests)	(91.3%)	(8.7%)	(65.2%)	(34.8%)	(65.2%)	(34.8%)
M3	24	2	20	6	20	6
(26 tests)	(92.3%)	(7.7%)	77%	23%	77%	23%
K82	17	3	19	1	16	4
(20 tests)	(85%)	(15%)	95%	5%	(80%)	(20%)
Total	110	12	89	33	86	36
(122 tests)	(90.1%)	(10.9%)	(72.9%)	(27.1%)	(70.5%)	(29.5%)

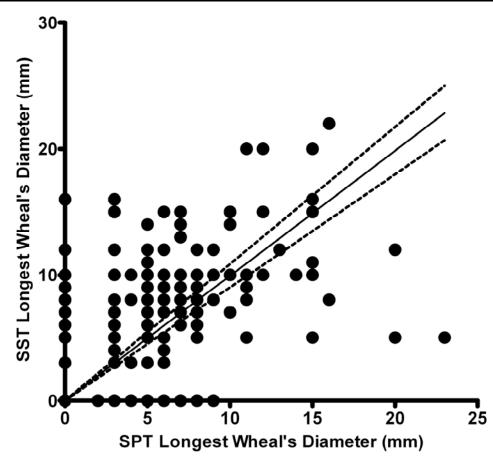


Fig. (2). Dispersion chart with 95% CI for the linear regression with 208 XY pairs between the skin scrape test (SST) wheal's longest diameters plotted against the skin prick test (SPT) wheal's longest diameters for airborne allergens in patients with s-IgE> 1.0 kU/L to corresponding allergens. Spearman r = 0.40 (0.27 to 0.51;p< 0.001).

of both tests analyzed together as a unique procedure was 70.5% (Table 4). Fig. (2) presents the dispersion chart with

95% CI of the linear regression with 208 XY pairs between the allergens SST WLD plotted against the allergens SPT

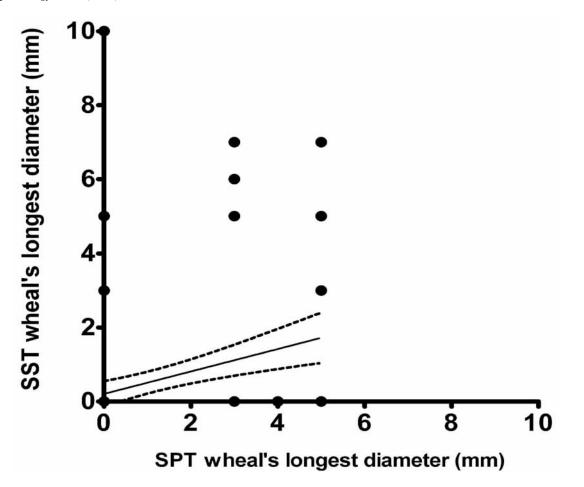


Fig. (3). Dispersion chart with 95% CI for the linear regression with 122 XY pairs between the skin scrape test (SST) wheal's longest diameters plotted against the skin prick test (SPT) wheal's longest diameters to airborne allergens in patients with s-IgE< 0.35 kU/L to corresponding allergens. Spearman r = 0.37 (0.20 to .51; p < 0.001).

WLD in patients with s-IgE> 1.0kU/mL to HDM (Spearman r = 0.40; 0.27 to 0.51; p < 0.001). Fig. (3) presents the dispersion chart with 95% CI of the linear regression with 122 XY pairs between the allergens SST WLD plotted against the allergens SPT WLD in patients with s-IgE< 0.35 kU/mL (Spearman r = 0.37; 0.20 to 0.51; p< 0.001). The difference of the allergens mean WLD between the SPT (6.0 mm) and the SST (7.6 mm) in patients with s-IgE> 1.0kU/L was 1.6 mm (0.88 to 2.21 mm; p < 0.001 by paired t test). The difference of the allergens mean WLD between the SPT (1.10 mm) and the SST (0.54 mm) in patients with s-IgE < 0.35kU/L was 0.56 mm (0.19 to 0.93 mm; p = 0.003 by paired t test). Differences between the allergens mean WLDSST between the group of patients with s-IgE>1.0kU/mL and the group with s-IgE < 0.35 kU/mL was 7.07 ± 0.44 mm (6.20 to 7.95 mm; p< 0.001 unpaired t test). Differences between the allergens mean WLDSPT between the group of patients with s-IgE>1.0kU/mL and the group with s-IgE< 0.35 kU/mL was $4.91 \pm 0.41 \text{ mm}$ (4.15 to 5.76 mm; p< 0.001 by unpaired t test - see Fig. 4).

DISCUSSION

There is no perfect exam that can identify every single allergen responsible for every single symptom in every single allergic patient. Even the s-IgE measurement has issues concerning differences in accuracy between distinct techniques, and these are somehow resolved by means of skin testing [20]. Therefore, a careful clinical history performed by an experienced physician remains the best way to establish a reasonable link between the results of skin or blood tests and allergic disease [21, 22]. A reactive skin test is a lead to be pursued by means of challenge tests to establish a diagnosis and to offer a proper therapy, or to remove the sources of the allergen [23]. In performing the SST, the skin is not scarified or broken. A needle's bevel is properly polished during its production in order to not present a reminiscent burr (unlike the primitive scarifies). The SST was perform to remove only the stratum corneum by sweeping the outermost skin layer that is largely responsible for the hydrophobic barrier function of the skin [24]. The stratum corneum has been described as a "brick-mortar wall" in which the terminally differentiated corneocytes (bricks) are embedded in the continuous matrix of specialized lipids (mortar). These lipids provide the essential element for the water barrier, while the corneocytes protect against physical injury [25]. Before the mid-1970s, the stratum corneum was thought to be biologically inert, but it is now appreciated to be both metabolically active and interactive with the underlying cell layers [26]. The simple removal of the impermeable barrier is enough to allow allergen penetration into adjacent skin layers without the need for deeper scarification. In order to remove the stra-

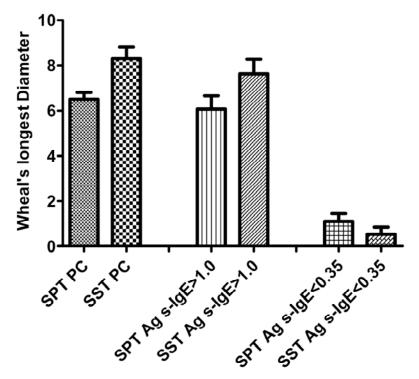


Fig. (4). Box column graphs with 95% CI whiskers plotted with the mean of the skin prick test (SPT) and the skin scrape test (SST) positive control (PC) mean wheal's longest diameter; SPT and SST allergens (Ag) mean wheal's longest diameter in patients with s-IgE> 1.0 kU/L; and the SPT and the SST allergens (Ag) mean wheal's longest diameter in patients with s-IgE< 0.35 kU/L.

Table 5. Comparative Features of Skin Scrape Test and Skin Prick Test

	Skin Prick Test	Skin Scrape Test	
Number of skin devices	One for each tested allergen	One for all tested allergens	
Discomfort	Pain full for toddlers and babies	Minimal tickles	
Dermographism	May be recognized only in negative control	May be recognized on each test before applying the allergens	
Sensitivity	86.5%	85%	
Specificity	72.9%	90.1%	

tum corneum as a preparation for patch testing for type IV allergy some centers routinely used tape stripping procedure with normal adhesive tape that is affixed and removed from the skin between one and ten times depending on the proband's skin type. We tried to perform this methodology, but it did not result in appreciable immediate skin reactions. We think that the residual glue left by the tape may diminishes skin permeability and be an interferent to the test.

The SSP was effective in demonstrating skin reactivity in some patients with the presence of s-IgE and who had a nonreactive SPT to HDM. On some occasions, we observed an inverse pattern with patients reacting to the SPT but not to the SST. Individual differences in the affinity threshold for IgE-allergen binding [27] may explain this phenomenon, as well the selective competition between immunoglobulins, which promote variable results to binding allergen affinity and skin tests [28]. For these reasons, some authors recommend performing an intradermic test to confirm a nonreactive SPT in a patient with a strong suggestive history of hypersensitivity to a particular allergen [29]. As the SST and the SPT are mutually complementary, the use of the other technique when the first does not react may spare the indication of the more traumatic and risky intradermic test. This is especially valuable when testing for food allergies because intradermic tests are usually not recommended for food allergens due to their association with an unacceptable rate of false-positive reactions [30]. The possibility of another "percutaneous" [31] technique that may be performed before the "intracutaneous" technique is very convenient because one can use the same allergen solution for the SPT and for the SST, while the intradermic test requires a non-glycerinated, more diluted and rigorously sterilized solution [9].

Another advantage of the SST is that it is less expensive than the SPT, as the health care professional may apply all antigens using a single hypodermic 18-G $1\frac{1}{2}$ needle available in most medical units (Table 5). To perform the SPT, one needs a disposable pricker for each tested antigen. In addition, the plastic dispenser saves the allergen solutions. With practice, the support provided by the plastic dispenser allows the health care professional to decrease the volume of the antigen solution applied to the skin. A standard drop applied directly with the dropper over the skin weighs an average of 0.035 g. The drop applied with the help of the dispenser weighs an average of 0.002 g. Despite the lower volume applied to the skin, there was no significant reduction in the wheal reading of the SPT or the SST. The wheals produced by the SST were approximately 2 mm longer than the wheals produced by the SPT (Table 1). In fact, most of the solution applied to the skin during the SPT and the SST was discarding after the test because only a tiny fraction of the solution reaches the mast cells. Squire estimated that during a SPT, only 0.000003 mL was introduced into the skin [32]. This reduction in costs may be a great advantage in public health facilities where allergy testing is limited by lack of resources [33].

CONCLUSIONS

The skin scrape test performed with the antigen dispenser is an inexpensive, painless and suitable technique to demonstrate IgE-specific sensitization with similar outcomes to the skin prick test. It may be used as an alternative duplicate test to the skin prick test or may even be used as a primary triage test, though it is subject to further confirmation by the skin prick test, the intradermic test, specific-IgE measurements and/or challenge tests.

CLINICAL IMPLICATIONS

The skin scrape test is a modified skin scratch test, without the pitfalls of the original technique, able to diagnose allergic sensitization with accuracy similar to the skin prick test.

CAPSULE SUMMARY

The skin scrape test is described in details. Sensitivity is defined in patients with IgE-mediated a topic conditions and specificity is defined with help of subjects with undetectable s-IgE to the studied antigens. The technique is compared with skin prick test.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

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