Skin Prick Automated Test device offers more reliable allergy test results compared to a manual skin prick test*

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Abstract

Background: The skin prick test (SPT) is the gold standard for identifying allergic sensitization in individuals suspected of inhalant allergy. A novel device, SPAT or Skin Prick Automated Test, that enables more standardized allergy testing has been developed. Previous research has shown reduced intra-subject variability of histamine wheals by SPAT.

Objective: This study aimed to evaluate within-test agreement (% of patients with consistent test results) to detect sensitization to common inhalant allergens when a SPT is executed automated by SPAT or by manual SPT (SPMT) procedure.

Methods: The 110 volunteers prospectively enrolled underwent both SPAT and SPMT with 3 pricks of house dust mite, timothy grass and birch, 2 pricks of histamine and 1 prick of glycerol. The proportion of consistent (3x positive – 3 x negative) and inconsistent (2x positive/negative – 1x positive/negative) test results were analysed.

Results: The proportion of inconsistent test results was significantly lower in the SPAT compared to the SPMT group. The delta histamine to control pricks was significantly higher in SPAT compared to SPMT group. Coefficient of variation was lower in SPAT compared to SPMT for house dust mite, timothy grass, birch pollen. Visual analogue scale for discomfort was significantly lower in SPAT compared to SPMT group.

Conclusion: SPAT showed a 34% reduction in the number of inconsistent test results compared to manual SPT with common inhalant allergens. Patient experience is significantly improved when an allergy test is performed by SPAT compared to a manual SPT.

Key words: Skin prick test, diagnosis, type I hypersensitivity, allergy, skin prick automated test, SPAT

Introduction

Respiratory allergies affect 30-40% of individuals worldwide and pose a major health-economic burden to society [(1)]. These numbers are considered to be an underestimation since it has been reported that 45% of patients remain undiagnosed because of lack of patient and doctor awareness or misdiagnosis [(2,3)]. Since effective therapies are widely available [(4)], a timely and correct diagnosis is crucial.

Evaluation of the atopic status in a patient suspected of an inhalant allergen is based on skin prick test or serum-specific IgE analysis [(5,6)]. Skin prick test (SPT) is the first choice diagnostic instrument according to international guidelines because of reduced cost, faster results, less invasiveness and better sensitivity-specificity profile compared to specific IgE [(7,8)].

Indeed, recently it has been shown that screening for sensitisation to inhalant allergens with in vitro molecular tests has lower sensitivity compared with extract-based skin tests [9].
Two-thirds of allergic diseases and 90% of respiratory allergies are diagnosed based on skin tests according to a European analysis of current clinical practice [10]. However, SPT exhibits both operator and device-dependent variability [11,12]. More specifically, insufficient prick depth and drug-related interference have been reported to lead to false-negative results. On the contrary, excessive prick pressure and the presence of skin disease, such as dermographism, may also lead to false-positive results.
Numerous device comparative studies have been performed, stating different outcomes for each one and advocating for continuous evaluation of these devices in their own practices settings [13–16]. These results highlight the need for standardization of the entire SPT procedure in order to limit human error, reduce variability and obtain more consistent and reliable test results.
To reach these goals, a novel skin prick automated test (SPAT) medical device has recently been developed. Performance, tolerability and safety of the device has been demonstrated before [17]. It was shown that SPAT exhibits reduced intra-subject variability compared to manual SPT when using control histamine and saline solutions.

In the current study, the within-test agreement of SPAT and skin prick manual test (SPMT) were compared for the most common inhalant allergens in Belgium. The level of discomfort of both tests has been assessed as a subjective parameter.

Materials and methods

Study design

A single-centre, prospective study was performed at the General Hospital of Herentals (AZ Herentals, Belgium) to evaluate within-test agreement to detect sensitization to common inhalant allergens with the SPAT device or by the SPMT procedure. Because the main objective of this study is to analyse reproducibility of positive and negative prick test results, it is preferable not to take seasonal differences [18] or the allergic status of the participants into account. The study was approved by the institutional review board and registered online at www.clinicaltrials.gov (NCT05824637).

Recruitment

Volunteers were recruited at the hospital of AZ Herentals via traditional communication channels (posters, information brochures) in March 2023. All study participants provided written informed consent before inclusion in the study. Volunteers irrespective of their atopic sensitization status between 18-65 years were eligible for inclusion in the study (Figure 1). The applied exclusion criteria were in line with the commonly used exclusion criteria of a SPMT [7]:

- Skin pathology like chronic or exuberant urticaria, dermographism, chronic dermatitis that needs daily treatment;
- Use of antihistaminic medication < 7 days before the start of the study;
- Use of tricyclic antidepressants (antihistamine activity) < 7 days before the start of the study;
- Use of topical corticosteroids on the forearm < 7 days before the start of the study;
- Use of Omalizumab < 6 months before the start of the study; or,
- Pregnancy.

SPAT and SPMT procedure

SPAT was carried out by use of a SPAT medical device (Hippo Dx, Aarschot, Belgium). SPMT has been executed as described in the European standard for SPT [7]. SPAT and SPMT were carried out by an experienced and trained nurse or clinician of the ear-nose-throat (ENT) department at AZ Herentals with supervision of one of the staff members of the ENT department. A new lancet (SPAT: Yilmaz Medikal, Gaziantep, Turkey; SPMT: ALK®, Horsholm, Denmark;) was used per individual prick.

Included participants underwent both the SPAT and the SPMT. SPAT was performed on the right arm and SPMT was performed on the left arm. On both arms, pricks were applied with dermatophagoides pteronyssinus (N=3 pricks), timothy grass (N=3) and birch (N=3), 10 mg/mL histamine (N=2) and glycerol-saline (N=1) as respectively positive and negative control (ALK, Hørsholm, Denmark). Allergen extracts used for either SPAT or SPMT were performed with the same production lots. For SPAT, the participant is asked to put their arm against the foreseen location of the SPAT device after the nurse or clinician started the testing procedure on the touch screen and the automated pricking procedure was started (Figure 1A). The SPAT applied twelve pricks simultaneously on the arm followed by a 90° clockwise rotation as described earlier [19].

The longest wheal diameter [20] of every test was measured for both SPMT and SPAT, both visually, on the spot, and digitally, through a SPAT recording. For the latter, 15 minutes after the first prick, 35 digital images were made with the SPAT device of both tested arms. These recordings were processed through an artificial intelligence system that generated a single overview or composite image. The on-the-spot readout was performed immediately after the digital imaging by a staff member of the ENT department. The digitally processed images were evaluated with specific SPAT software on a local network desktop by a staff member of the ENT department, one week after finishing all tests, and blinded for the prick test performed (Figure 1C-D).

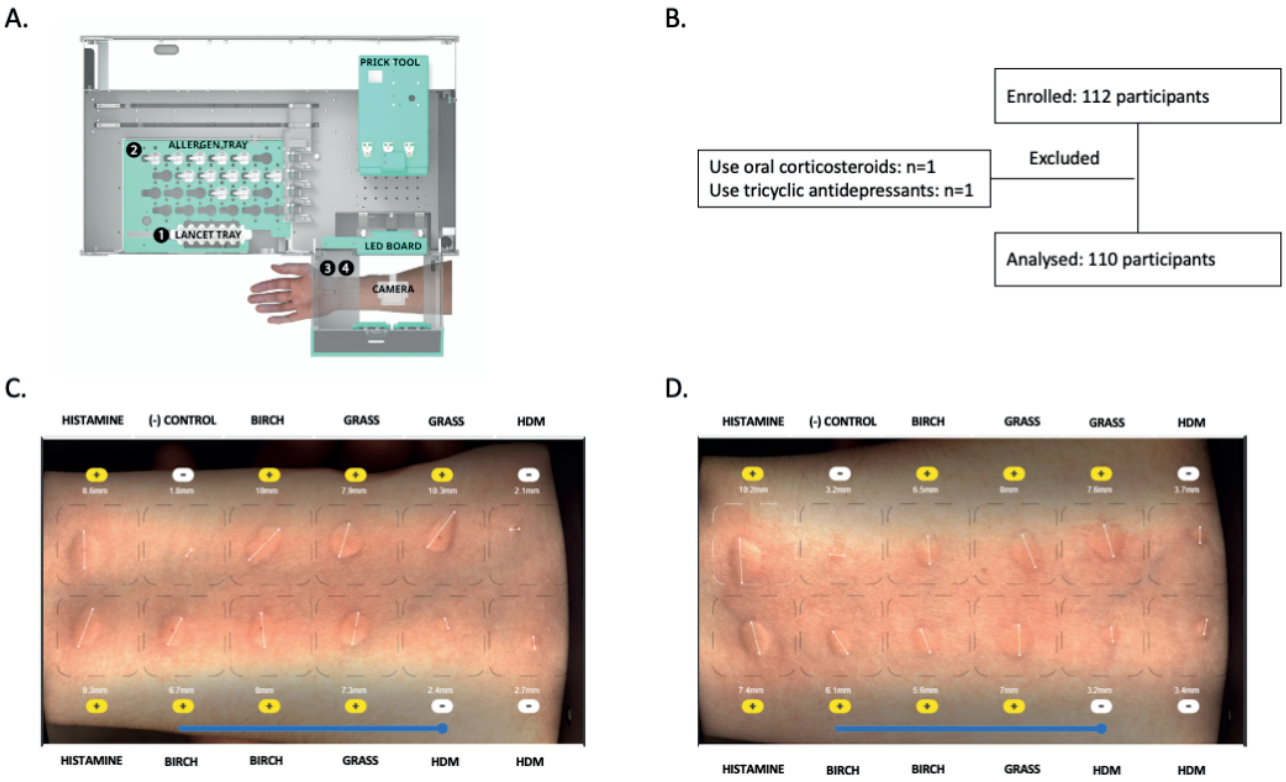

Figure 1. Study set up. A. Inside view of the SPAT medical device with the prick tool moving first to position (1), the lancet tray, to collect the lancets, then moving to position (2), the allergen tray, to collect the allergens from the vials and lastly moving to position (3), where the arm is positioned for the prick procedure. After 15 minutes, the arm is positioned at position (4), where the camera is also located, taking 35 images of the volar side of the arm. B. Data of 110 participants was analysed after excluding 2 participants who reported medication intake that may interfere with the skin prick test. C. Representative image of a skin prick manual test. D. Representative image of a skin prick automated test. HDM: house dust mite.

Safety

The skin prick test is safe with no reported fatalities in a 5-year USA study [21] and also adopted by the European guidelines on skin prick testing [7]. Because systemic allergic reactions might occur after skin prick testing, the study has been carried out similar to SPMT safety procedures, in a controlled environment on the ENT department of AZ Herentals in the presence of a clinician with emergency equipment present for the entire duration of the study. After the test, participants were asked to stay at least 20 minutes in the hospital before leaving. Adverse events were recorded during the course of the study.

Assessment of participant experience

A questionnaire was filled out by each participant to score the level of discomfort by visual analogue scale (VAS) after both SPMT and SPAT. The participants were asked to answer the questions on a 10 cm VAS from 0 ('not troublesome') to 10 ('worst thinkable troublesome').

Statistics

The proportion of consistent and inconsistent test results was compared between SPAT and SPMT by use of χ^2 test. The intra-subject coefficient of variation (CV) in wheal size is calculated as the standard deviation (SD) divided by the mean: CV = SD / μ [14,16,19]. Mann-Whitney test was used for between group comparison of non-parametric data. Statistical analysis was performed using Graphpad Prism 9 software.

Results

In total, 112 healthy volunteers were enrolled in the study (Figure 1B). Of these, 2 were excluded because of use of oral corticosteroids or tricyclic antidepressants in the week prior to the test. 110 health volunteers were analysed in the study (47 males – 63 females; mean ± standard deviation age: 41.6 ± 12.9). Six patients were identified as smokers. Subjects' clinical characteristics, including allergy symptom scores are represented in Table 1.

Within-test agreement

Within-test agreement was assessed by measuring the level of consistent and inconsistent test results. Three positive or negative test results with the inhalant allergens are considered consistent, one or two positive or negative test results are considered inconsistent. The proportion of inconsistent test results was

Table 1. Subject characteristics.

Number of study participants	110
Age (years)	41.6 ± 12.9
Male – Female	47 (42.7%) – 63 (57.3%)
Current smokers	6 (5.5%)
Visual Analogue Scale	
Total nose & eye symptoms	2.0 (0.0 – 7.0)
Rhinorrhoea	1.0 (0.0 – 6.0)
Nasal blockage	0.0 (0.0 – 5.0)
Itchy eyes	1.5 (0.0 – 7.0)
Itchy nose	0.0 (0.0 – 4.0)
Sneezing	1.0 (0.0 – 7.0)
Post-nasal drip	0.0 (0.0 – 2.0)
Cough	0.0 (0.0 – 2.0)
Dyspnoea	0.0 (0.0 – 1.0)
Reduced sense of smell	0.0 (0.0 – 2.0)
Facial pain	0.0 (0.0 – 4.0)

significantly lower in the SPAT (10.7%; 35/327 tests) compared to the SPMT (16.2%; 53/328 tests) group (p=0.04; Figure 2A). This corresponds to a 34% decrease in inconsistent test results with SPAT compared to SPMT (Figure 2B).

Test accuracy

False positive (positive glycerol saline prick) and false negative (negative histamine prick) test results were analysed. SPAT showed no false negatives (0/110 test results) compared to 10/110 false negative test results being identified with SPMT (p=0.001; Figure 3A). There was no significant difference in the number of false positive test results between SPAT (1/220) and SPMT (5/220) (p=0.10; Figure 3A). The ability to discriminate a positive from a negative test result was evaluated by analysing the wheal size difference between a histamine and a control prick. The delta histamine versus control was significantly higher in SPAT compared to SPMT test results (p=0.0001; Figure 3B).

Intra-subject variability of allergen pricks

SPAT showed lower coefficient of variation of the wheal sizes for respectively house dust mite, grass pollen and birch pollen (SPAT median (IQR): 13.1% (9.0% - 20.5%); 13.7% (8.9% - 23.0%); 15.0% (8.9% - 22.2%) compared to SPMT (SPMT median: 17.3% (11.2% - 24.4%); 17.4 (11.1% - 24.0%); 20.5% (11.8% - 28.6%) (p<0.0001; p=0.14; p=0.002; Figure 4A-C).

Assessment of participant experience

Subjective scoring of discomfort as assessed by VAS was significantly lower in the SPAT (median (IQR): 1 cm (0-2cm)) compared to the SPMT (2cm (0-3cm)) group (p=0.03; Figure 5).

Safety

No adverse events have been reported during the study for either test.

Discussion

In this study, we demonstrated a remarkable 34% reduction in the occurrence of inconsistent test results. Additionally, we observed significantly lower variability in test outcomes when utilizing the SPAT device for conducting skin prick tests with prevalent inhalant allergens, in contrast to manual administration by a trained nurse. Furthermore, the evaluation of patient discomfort revealed a more favorable experience with the SPAT device compared to the manual testing method.

In the absence of an allergy test that can be used as ground truth, which would require close to 100% specificity and sensitivity, validity of the SPAT device was evaluated by analysing the within-test agreement for a panel of common inhalant allergens. By demonstrating a significantly lower number of inconsistent test results with SPAT compared to the manual test approach, the primary outcome of the study was met.

As discussed before, conventional allergy testing exhibits variability because of device and operator dependent factors. It has indeed been shown that the outcome of the SPT result varies when different types of prick devices are used [14,16]. We previously demonstrated that the intra-subject variability of histamine wheals can be reduced by use of the S.P.A.T. medical device [17]. In the current study we could confirm these findings when SPT is performed with common inhalant allergens. Coefficient of variation (CoV) for house dust mite and birch pollen wheals was significantly lower in the SPAT compared to the SPMT group. An already low CoV value could potentially explain the lack of significance for grass pollen wheals. Literature review showed CoV with various devices ranging from 20 to 37% [14].

In line with our previous findings, larger wheals (longest wheal diameter) were detected after SPAT when compared to manual testing. A cut-off of 4.5mm was applied to determine positivity of the test result based on the 97.5th percentile of glycerol saline control wheal size [17]. This is in line with previous reports using bifurcated lancets for puncture [8]. Using this cut-off values for SPAT and the conventional 3.0mm cut-off for SPMT, false negative and false positive test results were analysed. The outcomes for false negative test results were in favour of SPAT whereas for false positive test results no significant difference could be detected. Interestingly, when analysing the ability of discriminating a positive from a negative wheal, SPAT showed a significantly higher delta histamine to glycerol saline control than SPMT.

When introducing a novel device for skin prick testing, it is also important to evaluate the patient experience. In this study we could confirm the significantly lower level of discomfort

A.

Allergens	SPAT	SPMT	Chi²
consistent	292	275	p= 0.04
inconsistent	35	53	

Positive test: ≥ 3.0 *mm for SPMT or* ≥ 4.5 *mm for SPAT*

Negative test: < 3.0 *mm for SPMT or* < 4.5 *mm for SPAT*

B.

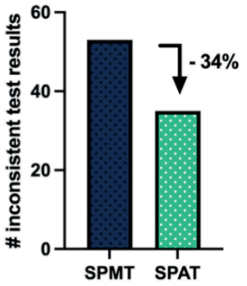

Figure 2. Within-test agreement. Study participants received a skin prick test by SPAT (right arm) or manually by a trained nurse (left arm) with 3 pricks of dermatophagoides pteronyssinus, timothy grass and birch, 2 pricks of histamine and 1 prick of glycerol saline. Three positive (≥3.0 mm for SPMT or≥4.5 mm for SPAT) or negative test results with the inhalant allergens are considered consistent, one or two positive or negative test results are considered inconsistent. Proportions are compared by Chi2 test.

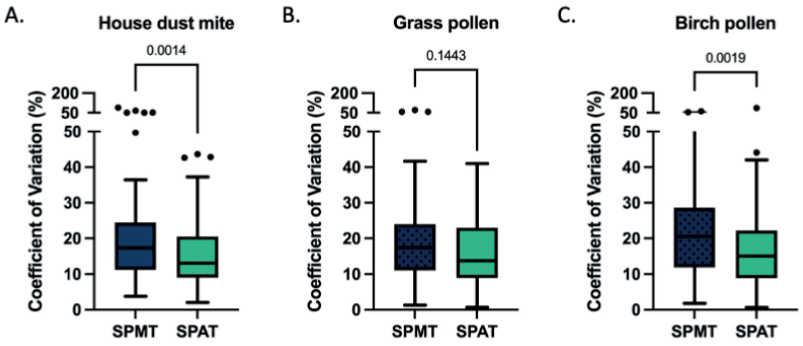

Figure 3. Test accuracy. A. The number of false positive (glycerol saline prick: ≥3.0 mm for SPMT or≥4.5 mm for SPAT) and false negative (histamine prick: <3.0 mm for SPMT or<4.5 mm for SPAT) were analysed. Proportions are compared by Chi2 test. B. The glycerol saline control wheal size was distracted from the average histamine wheal size to calculate the ability to distinguish a positive from a negative test result. In-between group comparison was performed with Mann-Whitney test. Data are represented as scatter dot plot with median and interquartile range.

A.

	SPAT	SPMT	Chi²
False negative	0	10	p= 0.001
True negative	110	100	

	SPAT	SPMT	Chi²
False positive	1	5	p= 0.10
True positive	219	215	

B.

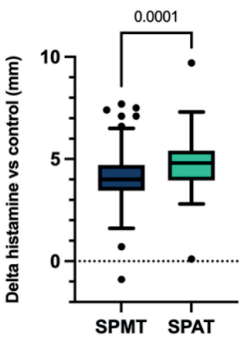

Figure 4. Intra-subject variability of allergen wheals. Coefficient of variation was calculated and compared between SPMT and SPAT by Mann-Whitney test. Data are represented as scatter dot plot with median and interquartile range.

reported by patients receiving a prick test via SPAT compared to a manual test. It is hypothesized that this is due to the 12 pricks being performed simultaneously in few seconds time. In comparison to other studies with average pain scores between 2-3 cm [22], the currently reported VAS scores (mean = 1.3 – median = 1.0) with SPAT are situated at the lower limit.

This is now the second study demonstrating more reproducible test results with the SPAT device. To validate the cut-off value to detect sensibilization to inhalant allergens, allergic and non-allergic individuals should be tested. In a next study, patients with a recent history of a positive and negative allergen provocation will therefore be evaluated. Whether the results obtained with SPAT are specific to the tested allergens is unclear. We were

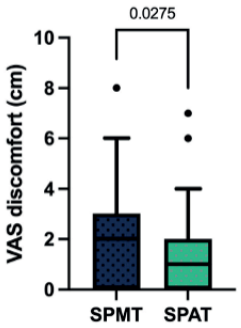

Figure 5. Subjective scoring of discomfort. Visual analogue scale (0 - 10 cm) was used to score the level of discomfort experienced by the participants. In-between group comparison was performed by Mann-Whitney test. Data are represented as scatter dot plots with median and interquartile range.

able to demonstrate reduced variability with histamine as well as 2 major inhalant allergens like house dust mite and birch but future studies with broader allergen panels should shed a light on that.

So far, no other medical devices combine automation of both the pricking and read out steps of the skin prick test procedure [23]. An SPT tape with 10 chambers each containing 3 micro-needles, thereby taking care of the prick procedure, has been evaluated before. The SPT tape showed equivalent accuracy to the manual SPT to detect patients with house dust mite sensitization [24]. Another device, ensuring digitalization of the SPT result, has been described in an exploratory trial. It was concluded that the agreement between the device and the manual procedure was moderate [25]. Other methods supporting the automated read out of the SPT results are based on 3D imaging [26] or are using a combination of visible-spectrum and thermal images [27]. The latter approach yielded rather good accuracy (93.6%) but is impacted by hair on the forearm as well as natural blood flow.

It should be noted that there are obviously also limitations to the study. Firstly, one cannot rule out potential technical issues with the use of a device. Secondly, the current study has been performed with 3 common inhalant allergens because of the assumption that variability and consistency outcomes are independent of the type of allergens. Lastly, SPAT was compared to standard clinical practice, in this case manual SPT with ALK lancets and not the lancets applied in the SPAT device. As such we cannot evaluate the relative contribution of the lancets and the device as a whole on the observed effects.

Conclusion

We were able to show that SPAT enables more consistent and less variable allergy testing compared to conventional methods of SPT. Such an approach contributes to standardisation of the SPT procedure by eliminating human error. From the patient perspective, SPAT is less uncomfortable for patients than the manual skin prick test.

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None.

Authorship contribution

SFS, MT, DL, HS, SG, LVG conceptualized and designed the study. KR, CC, LVC, LW, HS, SG contributed to the recruitment of volunteers and tests performed during the study. SFS, KR, SG, LVG contributed to the analysis and interpretation. SFS drafted the article, which was critically revised and edited by SG, LVG. All authors revised the manuscript and approved the final version.

Conflict of interest

MJT received consulting fees for statistical advice for the study. SFS, DL and SG are employees of Hippocreates. SFS, DL, SG and LVG hold shares of Hippocreates BV who developed the SPAT device. KR, CC, LVC, LW have nothing to disclose.

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