

High Histamine Control Concentration Leads to False Negative Allergy Skin Testing

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Abstract

Background: Allergy skin test reliability depends on the reagents and controls selected. Histamine is used at 1 mg/ml and 6 mg/ml concentration but few studies address the rationale for selecting one versus the other and how this may impact diagnostic accuracy.

Objective: To determine the rate of false negative allergen skin tests responses between UniTest PC (using the 1 mg/mL histamine) and Quintip devices (using 6 mg/mL) for 4 common aeroallergens.

Methods: Subjects aged 18–65 with symptoms of allergy to cat and/or ragweed received skin testing with 4 aeroallergens (dust mite mix, timothy grass, ragweed, cat), histamine and control diluent. Those individuals who tested positive to cat or ragweed with one skin prick test (SPT) device but not the other then proceeded to nasal allergen challenge (NAC). The primary outcomes were the aeroallergen false negative rates and sensitivities of the skin test devices followed by nasal allergen (NAC).

Results: Twenty-five individuals were recruited and underwent a total of 300 SPTs. SPT to allergens (ragweed, dust mite, cat, and timothy grass) resulted in a statistically significant difference in wheal size among the two skin testing devices (p value <0.0001 , 0.0001 , 0.0006 , and 0.0053 respectively). Six NAC procedures were performed to cat/ragweed and 5 of 6 (83% were positive). The overall allergen sensitivity rate for UniTest and Quintip were 97% and 78% respectively with most false negatives due to the use of 6 mg/ml histamine control reagent.

Conclusion: Our study shows that 6 mg/ml concentration of histamine control reagent may contribute to a false interpretation of aeroallergen skin prick test results.

Keywords

allergen, skin test, sensitivity, histamine, nasal allergen challenge, cat, ragweed

Introduction

For several decades skin prick testing (SPT) has remained a convenient, sensitive, and reproducible way to determine allergen sensitivity for the diagnosis of allergic disease such as allergic rhinitis, asthma and food allergy. Skin prick testing is also known to be very sensitive and specific when good technique is employed.^{1–3} Several groups have reported analytical sensitivity of greater than 95% and specificity of greater than 98% for devices using histamine as a stimulus.^{2–5} However, the reliability of skin prick testing to allergen extracts varies depending on several factors including the comparative accuracy of the positive and negative control reagents, quality of allergen extracts, characteristics of the skin test device used, and physiological factors.^{1,2}

Histamine positive control skin test reagents are typically used at 1 mg/ml and 6 mg/ml concentration but few studies address the rationale for selecting one versus the other and how this may impact the interpretation of allergen responses.

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The current study compares the rates of false negative allergen testing between two popular skin test devices namely, the UniTest PC and the Quintip. The primary objective of the study is to evaluate the performance of each device for the detection of sensitization to common aeroallergens and how this depends on control reagent selection. We report the rate of false negative allergen skin prick test responses between UniTest PC and Quintip devices for four common aeroallergens, contrast the results to the serum specific IgE levels, and evaluate the relationship between skin prick testing and graded nasal allergen challenge (NAC) for cat and ragweed.

Methods

Study Design

This was a prospective study to compare the diagnostic accuracy of two skin prick devices, Unitest PC (Lincoln Diagnostics, Decatur, Illinois) and Quintip (Hollister-Stier, Spokane, Washington) and reliance on control reagent selection. The study was conducted with approval by the Johns Hopkins Institutional Review Board. After potential study subjects were identified and written informed consent was obtained, participants were advised to stop taking any agents that may suppress skin prick test (SPT) for a period of at least 14 days, as well as nasal steroids, nasal antihistamines or other medications that may interfere with the nasal allergen challenge (NAC). After this washout period, blood was

drawn for specific IgE serology and then subjects received skin testing as previously reported and described in more detail below.^{4,6} Manufacturer recommended SPT techniques were performed using UniTest PC skin test device (Lincoln Diagnostics, Decatur, IL) and Quintip (Hollister-Stier, Spokane, WA) with 4 aeroallergens (dust mite mix containing *D. farinae* and *D. pteronyssinus*, timothy grass, ragweed, cat), histamine and control diluent. The allergen wheal and flare responses were measured and recorded by visual inspection, with a visible and short wave infrared camera (AllergyScope), and also manually by marking with a skin safe marker as per methods described previously.^{4,6}

Those individuals who tested positive to cat or ragweed with one SPT device but not the other then proceeded to NAC. The results of NAC were used to help determine the clinical relevance of a false negative allergen skin test. Intradermal testing was not performed as part of this study. Overall study design is shown in Figure 1.

Study Participants

Subjects aged 18–65 were recruited with allergy to cat and/or ragweed. Subjects were considered to be potentially allergic by report of symptoms or a history of positive testing to cat and/or ragweed pollen during the past two years. Patients with severe concurrent illnesses, uncontrolled asthma, extensive eczema, urticaria or dermatographism, pregnancy, using immunomodulatory

Study Design

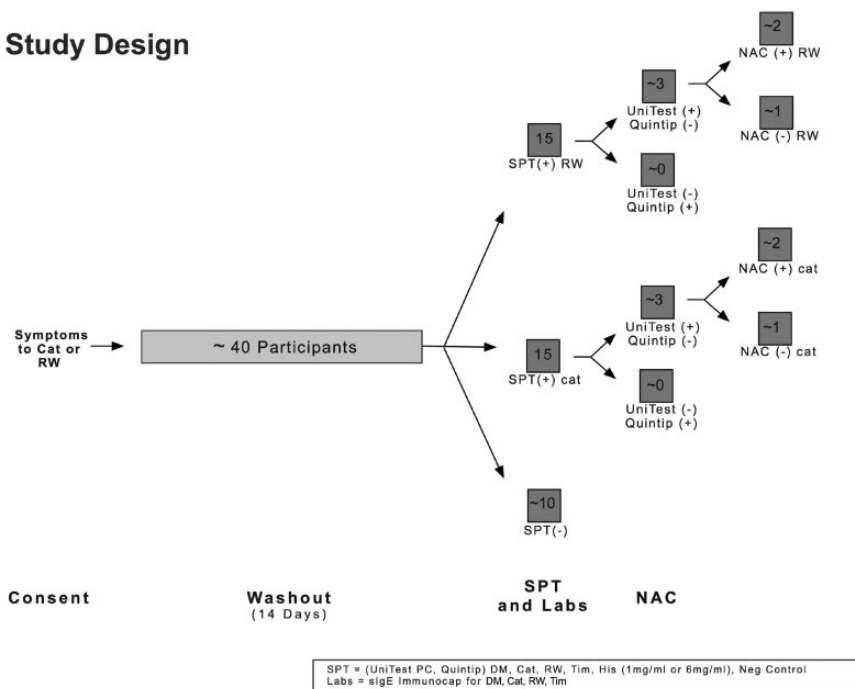


Figure 1. Study design.

drugs, using oral or topical steroids or allergen immunotherapy within the past three years were excluded. Those taking antihistamines, tricyclic-antidepressants, clonidine or sleeping aids as well as nasal steroids, nasal antihistamines or other medications that may interfere with the nasal allergen challenge (NAC) within 14 days before testing were also excluded.

Devices and Reagents

All skin test devices and reagents obtained for this study have been approved by the US Food and Drug Administration and were used with standard manufacturer recommended practices: UniTest PC skin test device, 6 mg/mL of histamine diluent (Hollister-Stier), 1 mg/mL of histamine diluent (ALK-Abello, Horsholm, Denmark), and 4 aeroallergens, dust mite mix, timothy, ragweed, and cat extract (Greer Laboratories, Lenoir, NC). All individuals received the same interventions, and the same reagents were used throughout the study.

Skin Test Protocol

Manufacturer recommendations were followed in the application of both devices for allergen skin testing with the UniTest device using 1 mg/ml histamine and Quintip device with 6 mg/ml histamine. The manufacturers instructions were reported previously.^{2,4,6} All individuals underwent a 14-day washout period during which they abstained from taking any agents that can suppress the SPT. On the study day, all individuals first received blood draw for the measurement of specific IgE to DM (Df and Dp), cat, timothy grass and ragweed using the ImmunoCAP system (Phadia, Upsala, Sweden) and then underwent standardized SPT testing on the forearm with 1 mg/ml histamine (UniTest PC manufacturer recommended positive control reagent) and 6 mg/mL (Quintip positive control reagent), control diluent, and 4 common aeroallergens (dust mite mix, timothy grass, ragweed, and cat). After 15 minutes, the wheal and flare responses were recorded by visual inspection and both a visible and short wave infrared camera (Allergyscope, Flare Diagnostics, Baltimore, MD).⁷ Wheal and flare were then circled with a skin-safe marker and transferred to paper using a strip of micro-pore tape. Wheal diameter was determined by a masked technician. Readings were verified by an independent investigator by using the skin test tracings and then further cross-checked by using AllergyScope images and computer assisted software.

Consistent with manufacturer instructions and previous publications,^{2,7,8} a SPT result was considered positive with a maximum wheal diameter that was at least 3 mm and simultaneously at least 2 mm more than the

negative control. An allergen test result was considered to be falsely negative when an allergen skin test result met criteria for positivity on one skin test device but was measured to be negative on the other skin test device. Results that were positive by serology but negative by both SPT devices were discounted similar to previous publications where SPT was used as the gold standard.^{9–15} If a false negative was identified by discordant SPT results with either cat or ragweed allergen, this was followed by a nasal allergen challenge (NAC) to one of the corresponding allergens in order to determine potential clinical relevancy.

NAC Protocol

Nasal allergen challenge (NAC) was performed as per standard practices described elsewhere.^{16,17} In brief, NAC consisted of receiving up to three concentrations of either ragweed or cat allergen with a graded, up-dosing using dilutions of extract of cat epithelium (Greer Labs 10,000 BAU/ml stock) or ragweed (Greer Labs ~200 Amb a 1/ml stock) which were diluted in normal saline to yield serial ten-fold dilutions of 5,000 BAU/ml (1:1), 500 BAU/ml (1:10), and 50 BAU/ml (1:100) cat and 25 Amb a 1/ml (1:1), 2.5 Amb a 1/ml (1:10), and 0.25 Amb a 1/ml (1:100) ragweed. Participants received two sprays of approximately 50 µl per nostril (200 µl total both nostrils) using a nasal applicator device (eg Aptar Pharma, Germany). Increasing concentrations of allergen were given every 10 min starting first with normal saline control and increasing sequentially to the top dose (1:1 dilution) if required to elicit symptoms. Immediately before each subsequent dose, participants were asked to grade their symptoms according to a validated scoring system: total nasal symptom score (TNSS), a 12 point scale with 4 categories: sneezing, nose running, nose blockage, and itching, each rated from 0 to 3 as reported by others.^{16,17} Once an individual achieved a TNSS score of 5 or more (and at least 2 points greater than baseline) then the NAC was considered positive and the remaining graded challenges were aborted. A TNSS of 4 or less at the final top dose was considered negative.

Statistical Analysis

Based on our previous studies and other published reports, we estimated that about 23% of subjects testing positive to cat or ragweed with the UniTest PC device would test negative to the Quintip skin test device. Using 95% confidence, a priori two-tailed power analysis was used to determine that a minimum sample size of 30 subjects (15 per group) would be required to yield statistical significance in detection of a false negative allergen test. An unpaired two-tailed t-test was used to

compare means and a linear regression model was used to obtain correlations. We performed a two-tailed, Fisher exact test to determine significance in the rates of false negative allergen testing between the two devices.

Results

About forty potential subjects volunteered for the study. Twenty-five individuals aged 18 to 65 years identifying with a history of allergic disease to cat or ragweed underwent a total of 300 SPTs (12 per individual). Consistent with prior publications, on average, the UniTest PC skin test device readings were larger than the Quintip readings ($P < 0.0001$). Placement of ragweed, dust mite mix, cat, and timothy grass, resulted in a statically significant difference among the two skin test devices in each of the corresponding wheal diameters observed ($P = 0.0001$, 0.0001 , 0.0006 , and 0.0053 respectively). Despite the statistically small wheal size among allergens tested in using the Quintip device, there was no difference in histamine responses between the two devices. Wheal diameter for histamine concentrations of 6 mg/mL and 1 mg/mL for the Quintip and UniTest skin testing devices respectively were statistically no different ($P = 0.6244$) as shown in Table 1. The overall sensitivity rates for UniTest, Quintip, and specific serum IgE testing were 97%, 77% and 73% respectively. Individual allergen sensitivity and false negative rates for RW, cat, dust mite, and

timothy grass were reported as well in Table 2. For cat and RW only, a total of 6 out of 32 SPT tests were falsely negative and all of these occurred using the Quintip device (Fisher exact $P = 0.024$). Of these false negative SPT, a total of six nasal allergen challenge (NAC) procedures were performed to either cat or RW. 5 of 6 (83%) NAC were positive indicating that the false negative allergen skin prick tests may have some clinical relevance.

Consistent with previously published reports, skin prick testing was significantly more sensitive than serum specific IgE measurements by ImmunoCap when SPT is used as the reference standard (Table 2).^{9-12,14,15,18-20}

AllergyScope derived SWIR images were approximately 1.5 mm smaller than manual measurements however the readings were very highly correlated ($P < 0.0001$) as seen in Table 3 and Figure 2.

Table 3. AllergyScope Derived SWIR Images Were Approximately 1.5 mm Smaller Than Manual Measurements However the Readings Were Very Highly Correlated ($P < 0.0001$).

	Manual SPT Readings	SWIR Images
Mean (SD)	6.47 mm (3.8)	5.03 mm (2.4)
N	91	91

P value < 0.0001 (delta 95% CI 0.9466–1.9424).

Table 1. SPT Wheal Diameter (mm).

Reagent	Saline	Histamine*	Ragweed	Cat	Dust Mite	Timothy
UniTest PC						
Mean (SD)	2.7 mm (2.1)	6.6 mm (1.6)	6.5 mm (3.3)	6.1 mm (3.7)	7.2 mm (3.5)	7.4 mm (5.1)
QUINTIP						
Mean (SD)	1.9 mm (1.9)	6.7 mm (1.9)	5.1 mm (3.2)	4.6 mm (3.0)	5.4 mm (3.0)	5.8 mm (4.4)
P value	0.1829	0.6244	< 0.0001	0.0001	< 0.0001	0.0053

*Quintip mean wheal diameter is significantly smaller for all aeroallergens but not the positive histamine control despite a higher concentration of histamine used with this device.

Table 2. False Negative Rate for the Each Test Method Is Shown.

Allergen	Overall	Ragweed	Cat	Dust Mite	Timothy
UniTest PC					
False negative	2/64	0/17	1/16	0/16	1/15
Sensitivity	97%	100%	94%	100%	93%
QUINTIP					
False negative	14/64	5/17	4/16	3/16	2/15
Sensitivity	78%	71%	75%	81%	87%
Serum IgE					
False negative	17/62	4/17	3/16	4/16	6/13
Sensitivity	73%	76%	81%	75%	54%

The overall sensitivity rate for UniTest, Quintip, and specific serum IgE were 97%, 78% and 73% respectively.

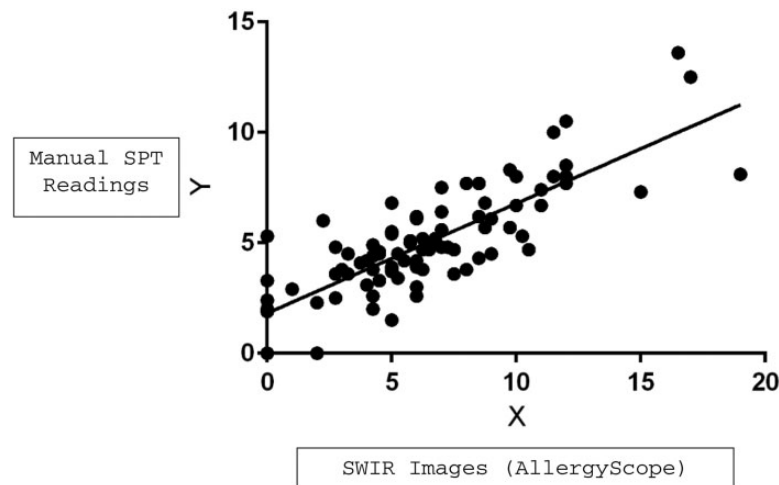


Figure 2. AllergyScope derived SWIR images are well correlated to manual SPT readings (mm) via linear regression model. P value < 0.0001, r value 0.7891, r square 0.6227.

Discussion

Quintip demonstrated a significantly higher rate of false negative tests for all aeroallergens tested. We believe this occurred in part because the 6 mg/ml histamine used as the manufacturer recommended positive control reagent is too highly concentrated. The higher concentration of histamine used for the Quintip device (6 mg/ml) yields exaggerated wheal responses compared to the aeroallergen-induced wheals. In the setting of possible exposure to antihistamines, the sensitivity of aeroallergen skin testing greatly depends on the presence of a positive control wheal, which may be falsely elevated by using a higher histamine concentration. Due to lingering antihistamine medication effects, the aeroallergens skin test response may be falsely negative, whereas if a lower histamine control concentration were used, the skin test would be negated. The consequence of high histamine control concentration is an inability to detect residual effects of antihistamines ultimately yielding more false negative aeroallergen results. In other words, too high concentration of histamine control may lead to a false confidence about the results whereby low-level allergen sensitivity may be missed.

The histamine control concentration used for the UniTest PC (1 mg/ml) yielded wheals that were comparable in size to the Quintip histamine wheals ($P = 0.6244$, however, the aeroallergen responses were significantly smaller with the Quintip ($P < 0.001$). This is because while the concentration of histamine used for the Quintip device was six times greater, the concentration of the allergen reagents remained the same for both devices. The result is more false negative allergen responses with the Quintip device.

One argument against the relevance of our results might be that the Quintip device does not suffer from

lower sensitivity but that the UniTest PC device is overly sensitive. We do not believe that to be the case in part because discrepant results were followed by positive allergen nasal challenge for cat and ragweed 83% of the time. The NAC results indicate that there may in fact be some clinical relevance to the higher SPT sensitivity by the UniTest PC device and 1 mg/ml histamine.

We recently published another study to determine the differential effect of cetirizine on two different concentrations of histamine and five common allergens used for percutaneous skin testing.⁶ This study similarly showed that the use of a 6 mg/mL histamine control for some percutaneous skin test devices may result in more false-negative allergen responses because of the inability to detect the presence of antihistamines. However, that study was not powered to expose the precise false negative rates for allergen testing. Our current study on the other hand, was specifically designed and powered to identify allergen false negative rates for both the UniTest PC (histamine concentration 1 mg/ml) and the Quintip (histamine concentration 6 mg/ml). We further compared skin prick testing, nasal allergen challenge (NAC), and serum specific IgE testing for ragweed and cat. False negative skin test results were similar for dust mite mix and timothy grass but were not followed by NAC for these two allergens due to practical limitations. We suspect that NAC results would be similar for these and other aeroallergens. It is yet to be determined if the differences in sensitivity we report for aeroallergens among the two devices would also be shown for food allergens or penicillin for example.

False negative allergen responses, while clinically significant for allergic rhinitis and asthma may have even more profound implications in the way we provide care for patients with food allergy and drug allergy.

The question of whether histamine concentration of 6 mg/mL is appropriate for testing should be revisited in these settings. Additional larger studies are required to help guide recommendations for appropriate use of this reagent from the FDA and other regulatory agencies.

Our results also demonstrate that both the UniTest PC and Quintip skin test device had a higher sensitivity compared to serum ImmunoCap measurement for the detection of aeroallergens overall. These findings should be interpreted with caution since our study was not designed to compare SPT to serology. As with many other studies, we used SPT as the gold standard to determine allergen sensitivity but this method has the potential to bias overall diagnostic accuracy in favor of SPT.

One limitation in this study is that we were not able to perform nasal allergen challenges (NAC) on all the aeroallergens we skin tested. We recommend further studies be performed to extend allergen challenge models to include timothy grass, dust mite, other aeroallergens, foods, and penicillin. These studies need to include serum IgE assays since several modern skin test devices have not been compared with current serological technologies. In the era of the COVID-19 pandemic, there has been a shift to increase serological testing offsite as an alternative to SPT, but the clinical significance to doing this may be relevant in terms of diagnostic potential.

In conclusion, our study shows that the concentration of histamine (i.e. 1 mg/mL for UniTest PC device and 6 mg/mL for Quintip device) may profoundly affect interpretation of allergen skin test results. Histamine concentration of 6 mg/mL increased the percentage of false negative allergen tests as confirmed by nasal allergen challenge. Larger clinical trials with confirmation by allergen challenge models are required to validate these findings.

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Declaration of Conflicting Interests

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