

Evaluation of three different histamine concentrations in intradermal testing of normal cats and attempted determination of 'irritant' threshold concentrations for 48 allergens

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Abstract

The purpose of this study was to determine the optimal histamine concentration and 'irritant' allergen threshold concentrations in intradermal testing (IDT) in normal cats. Thirty healthy cats were tested with three different histamine concentrations and four different concentrations of each allergen. The optimal histamine concentration was determined to be 1:50 000 w/v (0.05 mg mL⁻¹). Using this histamine concentration, the 'irritant' threshold concentration for most allergens was above the highest concentrations tested (4000 PNU mL⁻¹ for 41 allergens and 700 PNU mL⁻¹ for human dander). The 'irritant' threshold concentration for flea antigen was determined to be 1:750 w/v. More than 10% of the tested cats showed positive reactions to *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, housefly, mosquito and moth at every allergen concentration, which suggests that the 'irritant' threshold concentration for these allergens is below 1000 PNU mL⁻¹, the lowest allergen concentration tested. Our results confirm previous studies in indicating that allergen and histamine concentrations used in feline IDT may need to be revised.

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been sufficiently ruled out.^{1,2} Identification of the offending allergen(s) is critical for choosing the appropriate therapeutic approach, whether avoidance of the offending allergen(s) or immunotherapy (desensitization) is used.³ Currently, the same concentrations used in canine IDT are recommended in feline IDT.³⁻⁶ However, IDT in cats is still a subject of much controversy.^{1,4,5,7-12} The skin reactions in IDT in cats are often subtle and therefore difficult to interpret.⁷⁻¹² Many authors consider IDT in cats 'frustrating' or 'unrewarding'. In two studies, intravenous administration of fluorescein sodium during IDT aided in more accurate assessment of dermal reactions in normal and atopic cats.^{11,12} An additional problem in cats is the lack of internationally accepted standards for the concentration of the positive control, histamine, in IDT. In the USA, histamine is used at a concentration of 1:100 000 (0.01 mg mL⁻¹), whereas in Europe it is mostly applied at 1:10 000 (0.1 mg mL⁻¹).^{13,14}

The threshold concentration of an allergen used in IDT is defined in a standard veterinary dermatological textbook as the highest concentration that does not cause irritant reactions in at least 90% of normal individuals.¹⁴ Ideally, however, a given concentration of an allergen should cause no irritant reactions in normal cats and reliably discriminate between allergic and nonallergic individuals. Such an ideal allergen concentration would still leave the problem of subclinically sensitized cats showing positive reactions in IDT unaddressed. In order to get 'closer' to the ideal allergen concentration that causes no irritant reaction in normal cats, the 'irritant' threshold concentrations ('ITC') of allergens in normal cats need to be determined first.

Although optimal allergen concentrations for IDT in cats have not been well investigated, the following concentrations are commonly used: 1000 protein nitrogen units (PNU) mL⁻¹ for grasses, weeds, trees, moulds and insects; 250–500 PNU mL⁻¹ for feathers and epithelia; 250 PNU mL⁻¹ or 1:1000–1:5000 weight/volume (w/v) for individual house dust mites; and 1:1000 w/v for flea allergen.^{13,14}

The purpose of this study was to re-evaluate the histamine concentrations used in feline IDT and to determine the 'ITC' of allergens in normal cats.

Materials and methods

Cats

Thirty privately owned, clinically healthy cats with no history of skin problems were enrolled in the study. The cats had been in the current owners' households for at least 1 year. All owners gave informed written consent allowing their pets to be included in the study. The study

Introduction

Intradermal testing (IDT) is used in cats to support a tentative diagnosis of feline atopic dermatitis after other potential underlying diseases that cause pruritic dermatoses have

Allergen threshold based on	H1	H2	H3
1 Bahia grass	>4000	>4000	>4000
2 Bermuda grass	>4000	>4000	>4000
3 Blue grass/Kentucky	>4000	>4000	>4000
4 Fescue grass/Meadow	>4000	>4000	>4000
5 Johnson grass	>4000	>4000	>4000
6 Red top grass	>4000	>4000	>4000
7 Rye grass/perennial	>4000	>4000	>4000
8 Timothy grass	>4000	>4000	>4000
9 Cocklebur	>4000	>4000	>4000
10 Yellow dock	>4000	>4000	>4000
11 Dog fennel	>4000	>4000	>4000
12 Lambs quarter	>4000	>4000	>4000
13 Marsh elder/rough	≥4000	>4000	>4000
14 Pigweed rough	>4000	>4000	>4000
15 Plantain, English	>4000	>4000	>4000
16 Sorrel, red/sheep	>4000	>4000	>4000
17 Ragweed, southern	>4000	>4000	>4000
18 Ash, green/red	>4000	>4000	>4000
19 Birch, red	>4000	>4000	>4000
20 Cottonwood, eastern	>4000	>4000	>4000
21 Elm, American	>4000	>4000	>4000
22 Hickory, shagbark	>4000	>4000	>4000
23 Maple, red	>4000	>4000	>4000
24 Mulberry, red	>4000	>4000	>4000
25 Oak, Virginia/live	>4000	>4000	>4000
26 Sycamore, eastern	>4000	>4000	>4000
27 <i>Alternaria tenuis</i>	>4000	>4000	>4000
28 <i>Aspergillus niger</i>	>4000	>4000	>4000
29 <i>Curvularia spicifera</i>	>4000	>4000	>4000
30 <i>Fusarium moniliforme</i>	>4000	>4000	>4000
31 <i>Helminthosporium sativum</i>	>4000	>4000	>4000
32 <i>Hormodendrum hordei</i>	>4000	>4000	>4000
33 <i>Penicillium notatum</i>	>4000	>4000	>4000
34 <i>Pullularia pullulans</i>	>4000	>4000	>4000
35 <i>Dermatophagoides farinae</i>	<1000	<1000	<1000
36 <i>Dermatophagoides pteronyssinus</i>	<1000	<1000	=2000
37 <i>Tyrophagus putrescentiae</i>	≥4000	>4000	>4000
38 Human dander	>700	>700	>700
39 Dog epithelia	>4000	>4000	>4000
40 Sheep epithelia	≥4000	>4000	>4000
41 Duck feathers	>4000	>4000	>4000
42 Goose feathers	>4000	>4000	>4000
43 Flea	=1:250 w/v	<1:750 w/v	=1:750 w/v
44 Cockroach, American	=1000	>4000	>4000
45 Cockroach, German	>4000	>4000	>4000
46 House fly	<1000	<1000	=2000
47 Mosquito (Culicidae)	<1000	<1000	=2000
48 Moth	<1000	<1000	=1000

Table 1. Irritant allergen threshold concentrations based on different histamine concentrations

protocol was approved by the ethical committee/clinical research committee of the College of Veterinary Medicine of the University of Georgia. The group was composed of 16 spayed female and 14 neutered male cats. Their ages ranged between 2 and 8 years (mean age 4.5 years). There were 25 domestic shorthair cats, 2 domestic longhair cats, 2 Scottish Fold cats, and 1 Somali cat. Fifteen cats lived exclusively indoors, 10 lived exclusively outdoors, and five had an indoor/outdoor lifestyle. All the cats were on a high-quality monthly flea preventative year-round. No abnormalities were found upon physical examination. Complete blood cell counts, serum chemistry profiles, and urinalyses revealed results within the reference ranges. All cats tested negative for FIV (feline immunodeficiency virus) and FeLV (feline leukaemia virus).

Histamine phosphate (positive control) solution

Each cat was tested intradermally with three different concentrations of histamine phosphate solution (Greer Laboratories, Inc., Lenoir, NC, USA). The histamine concentrations were 1:100 000 (0.01 mg mL⁻¹) [H1]; 1:50 000 (0.05 mg mL⁻¹) [H2]; and 1:10 000 (0.1 mg mL⁻¹) [H3].

Phosphate-buffered saline (0.9%), containing 0.4% phenol as a preservative, was used for the dilutions. The histamine solutions were stored at 4 °C in glass vials for a maximum of 30 days.

Allergens

Forty-eight commercially available aqueous allergens were used for IDT (Table 1). The allergens included eight grasses (1–8), nine weeds (9–17), nine trees (18–26), eight moulds (27–34), three mites (35–37), five epithelia (38–42), and six insects (43–48). All allergens were obtained from Greer Laboratories, Inc., with the exception of the *Tyrophagus putrescentiae* test solution (storage mite), which was purchased from Center Laboratories (Port Washington, NY, USA). Each allergen was tested at four different concentrations. Phosphate-buffered saline (0.9%), containing 0.4% phenol as a preservative, was used for the dilutions. Forty-six of the 48 allergens were tested at 1000 PNU mL⁻¹, 2000 PNU mL⁻¹, 3000 PNU mL⁻¹, and 4000 PNU mL⁻¹. Flea allergen was tested at 1:750 w/v, 1:500 w/v, 1:250 w/v, and 1:100 w/v because no PNU concentration could be provided for the flea allergen by the manufacturer. Human dander was tested at 400 PNU mL⁻¹,

500 PNU mL⁻¹, 600 PNU mL⁻¹, and 700 PNU mL⁻¹ because the stock solution for this allergen was 700 PNU mL⁻¹. The diluted allergens were stored at 4 °C in glass vials for a maximum of 21 days.

Intradermal testing

The cats were sedated with 0.08 mg kg⁻¹ medetomidine (Domitor®, Pfizer, Exton, PA, USA) administered intramuscularly. The hair was gently clipped from the abdomen, flanks, and both sides of the thorax. The injection sites were drawn with a waterproof marker. The positive controls (H1, H2, and H3), the negative control [phosphate-buffered saline (0.9%) with 0.4% phenol], and 25 allergens (allergens 1–25, Table 1) at four different concentrations each, were injected intradermally on the left side of the body. The injection volume was 0.05 mL per injection, using 0.5-mL syringes with 27-G (0.5 mm) needles. Each injection site was evaluated objectively and subjectively at 15 and 30 min post-injection by the same investigator. Objective assessment consisted of measuring each wheal diameter, which was defined as the mean of the vertical and horizontal wheal diameters. Subjective assessment consisted of evaluation of erythema, turgidity, and depth of each wheal on a scale of 0–4+, with saline being equal to a zero (negative) reaction and histamine being equal to a 4+ reaction. After 30 min, the cats were turned laterally, the remaining dilutions of 23 allergens were injected, and the reactions were evaluated in the previously described manner. After 60 min, sedation was reversed by intramuscular injection of 0.4 mg kg⁻¹ atipamezole (Antisedan®, Pfizer, Exton, PA, USA).

A reaction to an allergen was considered positive if its diameter was equal or greater than the mean diameter of the negative control and positive histamine control (H1, H2, or H3). In addition, erythema and/or turgidity and/or depth of at least 1+ or greater had to be present for the wheal to be rated as a positive reaction. The 'I'TC for an allergen in IDT was defined as the concentration to which 10% of the normal cats (3/30 cats) showed a positive ('irritant') reaction.

Statistical analysis

Longitudinal data analysis on mixed model was used to test and compare the time and histamine concentration effects on the histamine wheal sizes.

The threshold concentration of each allergen was analysed in reference to the three different histamine concentrations using the chi-square test. All statistical comparisons were evaluated at a 5% level of significance ($P < 0.05$).

Results

Histamine phosphate (positive control) solution

The three different histamine concentrations resulted in positive reactions in all 30 cats. The mean wheal diameters of the H1, H2, and H3 wheals after 15 min were 10.6 mm, 12.7 mm, and 14.0 mm, respectively (ranges: 8–13 mm, 9–17 mm, 11–18 mm, respectively). The mean wheal diameters of the H1, H2, and H3 wheals after 30 min were 11.4 mm, 13.9 mm, and 15.6 mm, respectively (ranges: 8–15 mm, 11–18 mm, 12–20 mm, respectively). There were statistically significant differences in wheal sizes after 15 and 30 min within each histamine concentration ($P = 0.0003$ for H1, $P < 0.0001$ for H2, $P < 0.0001$ for H3) as well as between the different histamine concentrations ($P < 0.0001$ between H1 and H2, between H2 and H3, and between H1 and H3).

An H1 wheal size ≤ 10 mm was observed in 15/30 cats (50%) and 11/30 cats (36%) after 15 and 30 min, respectively. An H2 wheal size ≤ 10 mm was measured in only 2/30 cats and 0/30 cats after 15 and 30 min, respectively. An H3 wheal diameter ≤ 10 mm was present in only 1/30 cats and only after 15 min. In 8/30 cats (26%) the H3 reactions were subjectively more difficult to read than the H2 reac-

tions. Subjectively, the H2 wheals were easier to read than the H1 and H3 wheals.

'Irritant' allergen threshold concentrations

When H1 was used as the positive control, the 'I'TC for all tested grasses, weeds, trees, moulds, epithelia (with the exception of human dander), as well as German cockroach and *T. putrescentiae*, was at least 4000 PNU mL⁻¹ (Table 1). For human dander, the 'I'TC was at least 700 PNU mL⁻¹. For *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, house fly, mosquito, and moth, the 'I'TC was below 1000 PNU mL⁻¹. For American cockroach, the 'I'TC was determined to be 1000 PNU mL⁻¹. For flea allergen, the 'I'TC was found to be below 1:750 w/v.

When H2 was used as the positive control, the 'I'TC for all tested grasses, weeds, trees, moulds, epithelia (with the exception of human dander), as well as American cockroach, German cockroach, and *T. putrescentiae*, was at least 4000 PNU mL⁻¹ (Table 1). For human dander, the 'I'TC was at least 700 PNU mL⁻¹. For *D. farinae*, *D. pteronyssinus*, house fly, mosquito, and moth, the 'I'TC was below 1000 PNU mL⁻¹. For flea allergen, the 'I'TC was 1:750 w/v.

When H3 was used as the positive control, the 'I'TC for all tested grasses, weeds, trees, moulds, epithelia (with the exception of human dander), as well as American cockroach, German cockroach, and *T. putrescentiae*, was at least 4000 PNU mL⁻¹ (Table 1). For human dander, the 'I'TC was at least 700 PNU mL⁻¹. For *D. farinae*, the 'I'TC was below 1000 PNU mL⁻¹. The 'I'TC for *D. pteronyssinus*, house fly, and mosquito was determined to be 2000 PNU mL⁻¹. For flea allergen, the 'I'TC was 1:250 w/v. The 'I'TC for moth was 1000 PNU mL⁻¹.

No late-phase reactions were observed by the cat owners during a 24-h period post-IDT.

Discussion

No internationally accepted standard for the concentration of histamine, the positive control, in IDT exists to date. Our results show that different histamine concentrations have a significant impact on the wheal diameter of the positive control and also significantly influence the number of test allergen reactions rated as positive in IDT in normal cats. According to Reedy *et al.*, histamine reactions with a wheal diameter between 8 and 10 mm are not suitable in IDT.¹⁴ Fifty and 36% of our tested cats showed wheal diameters ≤ 10 mm after 15 and 30 min, respectively, when H1 (0.01 mg mL⁻¹ histamine) was injected, thus 'disqualifying' the H1 histamine concentration as a positive control in IDT in normal cats. Additionally, in 8/30 cats (26%) the wheal reactions of H3 were subjectively more difficult to read compared to the wheal reactions of H2 in the same individuals. The authors could not find a reasonable explanation for this phenomenon. Given the weak wheal reactions after injection of the H1 histamine concentration and the difficulties in reading the H3 reactions, using the H2 histamine concentration in feline IDT seems recommendable, although its usefulness in cats with atopic dermatitis will have to be a subject of future studies. A histamine concentration of 0.5 mg mL⁻¹ may seem high to some clinicians but in human dermatology, histamine concentrations ranging from 0.01 to 1 mg mL⁻¹ in IDT are commonly used.^{15–17}

The observation that many cats showed relatively small histamine wheal diameters 15 min post-injection, regardless of the histamine concentration, would suggest that evaluation 30 min post-injection is more recommendable. In some cats, however, the histamine wheal turgidity and/or depth and/or erythema 30 min post-injection was mildly weaker than 15 min post-injection. In order to consider these individual variations in cats undergoing IDT, evaluation of the positive control 15 and 30 min post-injection seems recommendable.

To the authors' knowledge, no comparable study has yet been conducted. In a study by Mueller *et al.*, different histamine concentrations ranging from 1:100 000 w/v to 1:9 600 000 w/v were tested in cats with a genetic predisposition for eosinophilic granuloma complex lesions.¹⁸ Histamine wheal diameters were subjectively graded from 0 to 4, with a mean wheal diameter of 11.3 mm in the strongest histamine group after 15 min. This mean wheal diameter is very similar to the mean wheal diameter of 11.4 mm in our healthy cats 30 min after the injection of H1.

To the authors' knowledge, only one study has been conducted in healthy cats to determine the 'ITCs for feline IDT using the same allergen source.⁶ This study included 50 cats and was conducted more than a decade ago using 24 allergens. All allergens, except for flea allergen, were tested at six different concentrations ranging from 25 to 1500 PNU mL⁻¹. Flea allergen was tested at six different dilutions ranging from 1:500 w/v to 1:4000 w/v. Histamine, at a concentration of 0.01 mg mL⁻¹, served as the positive control. The conclusion of the study was that allergen concentrations used in canine IDT are suitable for feline IDT, with the exception of firebush and flea antigen.⁶ The 'ITC for flea allergen was determined to be below 1:4000 w/v. These findings differ from the findings of the current study, although the allergens used in both studies were purchased from the same manufacturer (Greer Laboratories, Inc.). Differences in the results of both studies may be due to the variability in biological potency of allergens that can change from batch to batch within a short period of time and even more so within a longer period of time (like a decade) as standardization of allergens is suboptimal.^{14,15} Some results of the two studies are not directly comparable as firebush, house dust, and other allergen mixes were not used in our study. In the mentioned study, however, none of the cats reacted positively to sheep epithelium, sorrel red/sheep, *Alternaria tenuis*, *Helminthosporium*, or *Penicillium* allergen at the highest concentration of 1500 PNU mL⁻¹, which is similar to the findings in our study.

In a more recent study, 20 normal cats were tested with six different dilutions of *D. farinae* and *D. pteronyssinus* ranging from 1:100 w/v to 1:5000 w/v.¹⁹ None of the cats showed a positive reaction to any of the dilutions. The conclusion of the study was that allergen concentrations for dogs are probably not recommendable for cats. Histamine at a concentration of 0.01 mg mL⁻¹ served as the positive control. Although in this study the same allergen source was used as in our study (Greer Laboratories, Inc.), the results are not comparable because individual house dust mites were tested in PNU per millilitre in our study and not in w/v.

In a study with eight healthy cats, the author concludes that the threshold concentration for a mixture of *D. farinae* and *D. pteronyssinus* has to be greater than 1:1000 w/v as none of the tested cats showed positive reactions in IDT when histamine was used at a concentration of 0.01 mg mL⁻¹ for the positive control.²⁰ The fact that a house dust mite mixture was not used in our study prohibits the comparison of the two study results.

In the previously mentioned study, only 1/8 healthy cats reacted positively to flea allergen tested at a concentration of 1:1000 w/v. In another study with seven healthy cats, there were no positive reactions to flea antigen at a test concentration of 1:1000 w/v (Greer Laboratories, Inc.).²¹ Both studies used a histamine concentration of 0.01 mg mL⁻¹. In our study, we tested an even higher concentration of the flea antigen (1:750 w/v) and did not reach the 'ITC. All three studies used the same antigen source and came to the conclusion that the threshold concentration for flea allergen must be higher than 1:1000 w/v.

In a study conducted in Switzerland, 30 healthy cats were tested with allergen concentrations of up to 2500 PNU mL⁻¹.¹¹ None of the cats showed positive reactions at any of the tested concentrations. This study is not directly comparable to our study because a different allergen source was used (Allergopharma, Reinbek, Germany).

In a study conducted in the Netherlands, 10 healthy cats were tested with four different dilutions of 10 allergens.¹² The allergen concentrations ranged from 50 to 400 Noon units (NU) mL⁻¹ for *D. farinae*, and from 500 to 4000 NU mL⁻¹ in grass mix, mugwort, and cat flea. Only 0–1/9 cats showed a positive reaction to the highest allergen concentration tested for *D. farinae*, grass mixture, and mugwort. One to two of the nine cats reacted positively to any flea allergen concentration used in this study. The fact that the allergens used in this study were from a different manufacturer (ARTU Biologicals, Lelystad, the Netherlands) and quantified in a different way (NU) precludes comparison of the results to the results in our study.

Many authors have successfully used allergen concentrations recommended for canine IDT in determining cats with atopic dermatitis.^{12,22,23} The question whether potentially 'all' the cats with atopic dermatitis were identified with this approach remains unanswered, as well as the question whether all the normal cats were identified as such. The often weak and difficult to read reactions in feline IDT could, theoretically, either be due to the use of suboptimal allergen concentrations in this species, or they could simply be species-specific. Morris and Lindberg performed IDT in healthy horses and came to the conclusion that the recommended insect allergen concentrations in equine IDT may be too high as a significant number of the healthy horses showed an irritant reaction to these allergens in IDT.²⁴ As cats mainly show the opposite phenomenon concerning IDT results, the question of whether currently used allergen concentrations in feline IDT may be suboptimal seems legitimate.

In the vast majority of allergens tested in this study in healthy cats the 'ITC could not be determined. When using H1 or H2 as the positive control, the 'ITC for only one allergen could be determined. When using H3 as the positive control, the 'ITCs for five allergens were determined. For all other allergens, the 'ITCs were either

higher than the highest tested concentration or lower than the lowest tested concentration. Thus, further studies are needed to evaluate broader ranges of allergen concentrations in order to determine the exact 'ITCs for allergens in feline IDT in normal cats. This information can potentially be useful for better distinguishing 'irritant', nonallergic IDT reactions from true allergic reactions in cats with atopic dermatitis.

Ideally, a blinded controlled protocol should have been used for this study. However, except for the negative control and positive controls, the investigator did not know which allergens were injected until the test results were transferred to a table after the testing. For this reason, we believe that the results of our study are still of scientific value.

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Résumé Le but de cette étude était de déterminer la concentration optimale d'histamine et les concentrations 'irritantes' d'allergènes pour réaliser des tests intradermiques (IDT) chez le chat normal. Trente chats sains ont été testés avec trois concentrations différentes d'histamine et quatre concentrations différentes de chaque allergène. La concentration optimale en histamine était de 1:50 000 w/v (0.05 mg/mL). En utilisant cette concentration d'histamine, la concentration «irritante» pour la plupart des allergènes était supérieure aux concentrations les plus fortes utilisées (4000 PNU/ml pour 41 allergènes et 700 PNU/ml pour les squames humaines). La concentration 'irritante' pour la puce était de 1:750 w/v. Plus de 10% des chats testés étaient positifs pour *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, la mouche, le moustique à chaque concentration testée, ce qui suggère que la concentration «irritante» pour ces allergènes est inférieure à 1000 PNU/ml, la plus faible concentration allergénique testée. Nos résultats confirment les études précédentes indiquant que les concentrations d'allergènes utilisées dans l'espèce féline pour les IDT pourraient être révisées.

Resumen El propósito de este estudio fue determinar la concentración óptima de histamina y el nivel mínimo de alérgeno 'irritante' en la prueba intradérmica (IDT) en gatos normales. Treinta gatos en buen

estado de salud se sometieron a una prueba con tres concentraciones de histamina y cuatro de alérgeno. La concentración óptima de histamina se determinó en un valor de 1:50 000 m/v (0.05 mg/ml). Utilizando esta concentración de histamina, el nivel mínimo de 'irritante' para la mayoría de alérgenos estuvo por encima de las mayores concentraciones utilizadas (4000 PNU/ml para 41 alérgenos y 700 PNU/ml para la descamación procedente de humanos). El nivel mínimo de irritante para el antígeno de las pulgas fue determinado en un valor de 1:750 m/v. Más del 10% de los gatos en las pruebas demostraron reacciones positivas a *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, mosca doméstica, mosquito, y polillas a todas las concentraciones de alérgeno, lo que sugiere que el nivel mínimo 'irritante' para estos alérgenos está por debajo de 1000 PNU/ml, la menor concentración de alérgeno en las pruebas. Nuestros resultados confirman estudios previos que indicaban que las concentraciones de alérgeno e histamina utilizadas en la prueba intradérmica felina deberían ser revisadas.

Zusammenfassung Das Ziel dieser Studie war es, die optimale Histaminkonzentration und einen Grenzwert für 'irritierende' allergische Konzentrationen beim Intradermaltest (IDT) bei normalen Katzen festzulegen. Dreißig gesunde Katzen wurden mit drei verschiedenen Histaminkonzentrationen und vier verschiedenen Konzentrationen eines jeden Allergens getestet. Die optimale Histaminkonzentration wurde mit 1:50 000 w/v (0.05 mg/ml) festgelegt. Bei Verwendung dieser Histaminkonzentration lag der Grenzwert für die 'irritierenden' Konzentrationen der meisten Allergene über den höchsten Konzentrationen, die getestet wurden (4000 PNU/ml für 41 Allergene und 700 PNU/ml für menschliche Hautschuppen). Der Grenzwert für die 'irritierende' Konzentration von Flohantigen wurde mit 1:750 w/v festgelegt. Mehr als 10% der getesteten Katzen zeigten positive Reaktionen auf *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, Stubenfliege, Moskito und Motte bei jeder getesteten Allergenkonzentration, was darauf schließen lässt, dass der Grenzwert für die 'irritierenden' Konzentrationen dieser Allergene unter 1000 PNU/ml liegt, welches die niedrigste getestete Konzentration darstellte. Unsere Ergebnisse bestätigen jene früherer Studien, indem sie darauf hinweisen, dass die Allergen- und Histaminkonzentrationen, die beim feline IDT Verwendung finden, möglicherweise überarbeitet werden müssen.