

Determination of irritant threshold concentrations to weeds, trees and grasses through serial dilutions in intradermal testing on healthy clinically nonallergic dogs

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Conflict of Interest

No conflicts of interest have been declared.

Abstract

Irritant threshold concentration (ITC) for intradermal testing (IDT) was determined in 31 healthy, clinically nonallergic dogs. Twenty-three allergens were tested at five variable concentrations ranging from 1000 to 8000 PNU/mL. To distinguish irritant reactions from subclinical IgE-mediated hypersensitivities, serum allergy testing was performed. ITCs were determined by evaluating the lowest concentration to which no dogs (0% cut-off) and to which at least 10% of dogs ($\geq 10\%$ cut-off) reacted. ITCs at the 0% cut-off were: 1000 PNU/mL (Johnson grass), 2000 PNU/mL (Ash, Lamb's Quarter and Bermuda), 3000 PNU/mL (Bahia, Rye, Pig Weed and Virginia Oak), 4000 PNU/mL (Marsh Elder and Maple), 5000 PNU/mL (Sorrel sheep) and 7000 PNU/mL (Cocklebur and Black Willow). ITC for Dog Fennel, Box Elder and Red Cedar was <1000 PNU/mL. ITCs at the $\geq 10\%$ cut-off were: 2500 PNU/mL (Johnson), 3000 PNU/mL (Box Elder), 5000 PNU/mL (Bahia), 6000 PNU/mL (Pigweed and Marsh Elder) and 8000 PNU/mL (Virginia Oak and Black Willow). For all other allergens, the ITC was >8000 PNU/mL and could not be determined. No significant agreement between positive values was found for the same allergen on IDT and serum allergy testing for each dog suggesting reactions caused by the determined ITCs are less likely subclinical IgE-mediated reactions. These results suggest that ITCs

may vary, also they may be very high for the allergens tested and that higher test concentrations may be used for IDT for the tested allergens without inducing an irritant reaction. Further studies are needed to evaluate the benefit of higher IDT concentrations in atopic dogs.

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Introduction

Canine atopic dermatitis is a common disease afflicting dogs. Appropriate allergy testing in atopic pets is crucial, giving pet owners a specific treatment for their pets through allergy avoidance and allergen specific immunotherapy.¹ Intradermal testing (IDT) is a method of allergy testing used to confirm the diagnosis of atopy and identify the responsible IgE-mediated allergen hypersensitivities.^{1–3} For IDT, false-positive reactions may be defined as reactions observed at the IDT site that resemble the erythematous wheal of a typical IgE-mediated reaction to allergen, but that are not IgE-mediated.³ Allergen-specific IgE serum testing (ASIST) is a method used to demonstrate IgE-mediated hypersensitivity.⁴

In principle, allergen extracts used for IDT should be tested at a threshold concentration, or the highest concentration of the extract that does not cause an irritant reaction that may be interpreted as a false-positive reaction.³ The current method for establishing the threshold concentration is through testing serial allergen dilutions on normal, nonatopic dogs and adjusting the concentration so that no more than 10% of normal dogs have positive reactions, and then to test the new concentrations on diagnosed atopic dogs.^{1,2}

The typical allergy dilution recommended and generally used by most dermatologists for IDT for pollens of trees, grasses and weeds is 1000 PNU/mL,³ however, some obviously atopic dogs can have a negative IDT. Anecdotally some dermatologists use higher test concentrations. Recent data suggest that using concentrations at 1750 PNU/mL for plant allergens did not produce an irritant reaction in tested healthy normal dogs.⁵ The purpose of this study was to determine the irritant false-positive threshold concentration (ITC), through serial dilutions, using IDT in 31 healthy, clinically nonallergic dogs.

Materials and methods

Study design

This was a prospective, blinded-controlled study. The study protocol and owner consent form were approved by the Clinical Research Committee of the College of Veterinary Medicine of the University of Georgia.

Dogs

Thirty-one privately owned (staff and students), clinically healthy dogs were used in this study. They included 15 neutered males, 13 spayed females, two intact males and one intact female. Their ages ranged from 1.5 to 8 years (mean age 4.75 years) and weights ranged from 7.8 to 50 kg (mean weight 28.9 kg). There were 16 mix-breed dogs, five German shepherd dogs, two golden retrievers, one Labrador retrievers, one boxer and one each of American Eskimo, Australian shepherd, Bernese mountain dog, Japanese chin, malamute and rottweiler. The dogs had been in the current owners' households for at least 1 year and none of the dogs had any history of pruritus or skin and ear diseases.

No abnormalities were found on the general physical examination, dermatologic examination, hemograms, serum chemistry profiles, or urinalysis. Thyroxine levels were within normal limits for all 31 dogs. All animals were current on routine vaccinations and were receiving monthly flea and heartworm preventative.

Allergens

Twenty-three commercially available aqueous allergens were used for IDT. The allergens included seven grasses (1–7), seven weeds (8–14) and nine trees (15–23) (Table 1). All allergens were obtained from Greer Laboratories Inc. Dogs were tested in three groups. The numbers of dogs among test groups were distributed as followed: group 1 ($n = 14$), group 2 ($n = 6$) and group 3 ($n = 11$). The allergen concentrations tested in group two were based on the results of group 1 and the test concentrations for the group 3 IDT were based on the skin test results of group 1 and 2 (Table 2). For the test, each allergen was diluted with phosphate buffered saline to obtain five different test concentrations. All allergens in the first group were tested at the following concentrations: 1000, 2000, 3000, 4000 and 5000 PNU/mL. Allergens in the second and third groups were tested at the following concentrations 4000, 5000, 6000, 7000 and 8000 PNU/mL except Johnson grass, Box Elder and Red Cedar. For the second and third groups Johnson grass was tested at 2000, 2500, 3000 3500 and 4000 PNU/mL; and then 1500, 1750, 2000, 2500 and 2750 PNU/mL, respectively. For the second and third groups Box Elder was tested at 1000, 1250, 1500, 1750, 2000 PNU/mL and then 1000, 2000, 3000, 4000, 5000 PNU/mL, respectively. For the second and third groups Red Cedar was tested at 1000, 3000, 4000, 5000, 6000 PNU/mL. The diluted allergens were stored in glass vials at 4 °C and diluted prior to each group tested. Once allergens diluted, the second investigator blinded and double randomized the vials prior to the first investigator performing and evaluating the IDT.

Allergy-specific IgE serum testing

Serum samples (1.5–2 mL) were obtained and submitted for an allergy-specific IgE ELISA test using a recombinant α -chain of the high-

affinity IgE receptor (Fc ϵ R1 α). A modified Heska® Allerecept® (Fribourg, Switzerland) test was used evaluating all allergens used for IDT in this study. ELISA Absorption Units (EA) of 150 or higher was considered positive.

Intradermal testing

All testing was performed and objectively evaluated by the first investigator. The dogs were prepared for testing using medetomidine 20–40 μ g/kg intravenously (Domitor®, Pfizer, Exton, PA, USA) for sedation. The skin over the left ventro-lateral thorax wall was carefully clipped using a #40 clipper blade. The skin test sites were marked with a waterproof marker. Each dog was then injected intradermally with 0.05 mL of histamine solution 1 : 10 000 (0.1 mg/mL) as the positive control, a negative control solution (0.9% phosphate buffered saline) and 115 allergen solutions (23 allergens at five different concentrations) using 0.5-mL syringes and 27-G (0.5 mm) needles. Each skin test site was evaluated at 15- and 30-min post-injection by measuring the diameter (average of the vertical and horizontal diameter) of any reaction. The negative and positive controls were evaluated at 15 min for determination of the diameter to which the allergen reactions would be compared. A reaction of an allergen test concentration was considered positive when the diameter of any wheal and/or erythema formation was equal to or greater than the mean diameter between the negative control and the positive histamine phosphate control being measured.

Statistical analysis

Descriptive statistical analysis was used to evaluate two allergen threshold concentrations. We defined $\geq 10\%$ cut-off ITC as the concentration where greater than or equal to 10% of the total number of dogs tested at that concentration showed positive reactions. In addition we defined $\leq 0\%$ cut-off ITC as the concentration where 0% of the total number of dogs tested at that concentration showed positive reactions.

Analysis to compare serology and IDT results were evaluated using kappa statistics. The SAS v 9.1 (Cary, NC, USA) computer program was used for analysis. Kappa statistics was used to test for agreement of serology and IDT results for all 23 allergens for each dog separately.

In addition, a two-sided binomial test was used to compare the percentage of skin test positive results that were also blood test positive to 10% which would be expected if there was no relationship between blood and skin test results. The results from all 23 allergens were pooled for analysis. The 10% was used as a default baseline for blood test positive rate and was derived from both the overall blood test positive rate for all dogs with all allergens pooled ($78/759 = 10\%$) and the blood test positive rate for all dogs that had a negative skin test result ($71/698 = 10\%$).

Results

Allergy-specific IgE serum testing

The values of positive results ranged from 151 to 2888 EA units. Four allergens (Willow, Red Cedar, Ash and Box Elder) had no positive results on the ASIST. Rye

Table 1. Allergens used for IDT in this study

Grasses	Weeds	Trees
1. Bahia Grass	8. Cocklebur	15. Ash, Red/Green
2. Bermuda Grass	9. Dog Fennel	16. Box Elder
3. Blue Kentucky/June	10. Lamb's Quarter	17. Hickory, Shagbark
4. Fescue Grass, Meadow	11. Marsh Elder, True/Rough	18. Maple, Red
5. Johnson Grass	12. Pigweed, Rough/Redroot	19. Mulberry, Red
6. Rye, Perennial	13. Ragweed, Southern	20. Oak, Virginia live
7. Timothy Grass	14. Sorrel, Sheep/Red	21. Red Cedar
		22. Sycamore American/Eastern
		23. Willow, Black

Table 2. Data showing the total number of dogs tested at each concentration for each allergen

1-4,12-15, 17-23 (PNU/mL)	Tested*	Johnson grass (PNU/mL)	Tested*	Box Elder (PNU/mL)	Tested*	Red Cedar (PNU/mL)	Tested*
1000	13	1000	13	1000	31	1000	31
2000	13	1500	11	1250	7	2000	13
3000	13	1750	11	1500	7	3000	31
4000	31	2000	31	1750	7	4000	31
5000	31	2500	18	2000	31	5000	31
6000	18	2750	11	3000	24	6000	18
7000	18	3000	20	4000	24		
8000	18	3500	7	5000	24		
		4000	20				
		5000	13				

*Tested refers to the total number of dogs tested.

grass, Johnson grass and Fescue had the highest values of 2888, 2782 and 2802, respectively. Of these dogs with test results amenable for evaluation, the kappa statistics was not significantly different than 0, meaning the IDT and ASIST were not in agreement significantly more than would be expected by chance. Using the two-sided binomial test, of the 61 skin test positive results 7% or 11.5% were also blood test positive which was not significantly different than 10% ($P = 0.8210$).

Intradermal testing

The $\geq 10\%$ cut-off to establish ITCs could be determined in Bahia grass at 5000 PNU/mL, Johnson grass at 2500 PNU/mL, Marsh Elder/Rough at 6000 PNU/mL, Pigweed, rough at 6000 PNU/mL, Box Elder at 3000 PNU/mL and Black Willow at 8000 PNU/mL. For all other allergens tested the irritant threshold concentration (ITC) could not be determined and was least 8000 PNU/mL. Thirteen ITCs were identified using a 0% cut-off which determines an ITC where the highest concentration with 0 healthy dogs reacted at this concentration and all lower concentrations tested and were as follows: Johnson grass at 1000 PNU/mL, Bermuda, Lamb's Quarter and Ash Red/Green at 2000 PNU/mL, Bahia grass, Rye/Perennial, Pigweed and Virginia Oak at 3000 PNU/mL, Marsh Elder and Maple Red at 4000 PNU/mL, Sorrel/Sheep at 5000 PNU/mL and Cocklebur and Willow Black at 7000 PNU/mL. For all other allergens tested the ITC could not be determined and was either greater than 8000 PNU/mL, the highest concentration tested, or at least one dog tested reacted to every concentration tested defined as lesser than 1000 PNU/mL (Table 3–5).

Table 3. ITC for grasses

Grasses	0%	$\geq 10\%$
Bahia	3000	5000
Bermuda	2000	>8000
Blue Kentucky	>8000	>8000
Fescue Meadow	>8000	>8000
Johnson	1000	2500
Rye Perennial	3000	>8000
Timothy	>8000	>8000

0% and $\geq 10\%$ values are expressed as PNU/mL.

Table 4. ITC for weeds

Weeds	0%	$\geq 10\%$
Cocklebur	7000	>8000
Dog Fennel	<1000	>8000
Lamb's quarter	2000	>8000
Marsh Elder, Rough	4000	6000
Pigweed	3000	6000
Ragweed, Southern	>8000	>8000
Sorrel, Sheep	5000	>8000

0% and $\geq 10\%$ values are expressed as PNU/mL.

Table 5. ITC for trees

Trees	0%	$\geq 10\%$
Ashe, Red/Green	2000	>8000
Box Elder	<1000	3000
Hickory, Shagbark	>8000	>8000
Maple, Red	4000	>8000
Mulberry, Red	>8000	>8000
Oak, Virginia Live	3000	8000
Red Cedar	<1000	>8000
Sycamore American/Eastern	>8000	>8000
Willow Black	7000	8000

0% and $\geq 10\%$ values are expressed as PNU/mL.

Discussion

The differentiation of subclinical allergies from 'false'-positive irritant reactions in dogs can be challenging, but an attempt to do so was important when utilizing clinically 'normal' animals for this study. Allergies are associated most commonly with the development of IgE hypersensitivities.⁶ Using the Heska Allercept™ Detection System, *in vitro* measurement of canine IgE was performed. This test was used to determine if any of the study participants would show an allergen-specific IgE reaction indicating a possible subclinical allergy making any corresponding positive reactions on IDT likely true positive reactions, and not irritant (false-positive) reactions. Out of a total of 31 dogs tested only four had overlapping test results and out of 713 possible overlapping reactions there were only a total of seven instances where there was a positive IDT and serum allergy test (Table 6). Based on the lack of significant agreement between the serology test results and the IDT results it was determined that the dogs in this study were unlikely to be subclinically

Table 6. Concurrent serum positive and IDT positive dogs

Dog*	Bahia	Bermuda	Johnson grass	Pigweed	Dog Fennel	Mars Elder
1	5000 PNU/mL	206 EA				
3			2500, 4000 PNU/mL	2782 EA		
7		3000 PNU/mL	243 EA		1000, 4000 PNU/mL	248 EA
28			2750 PNU/mL	582 EA	4000, 7000 PNU/mL	198 EA
						7000, 8000 PNU/mL
						275 EA

*Number assigned to each dog out of a total of 31 dogs.

Numbers given are the IDT PNU/mL compared with the EA units found on the Allercept® results.

allergic and therefore considered 'normal healthy' dogs for establishing threshold concentrations. While the purpose of this study was not to compare serum allergy testing and IDT, one reason for the difference in positive results found by the serum allergy test and IDT could be that the dogs tested are nonallergic dogs. Therefore, any coincidental positive reactions were within statistical expectations. Another possibility is the allergens used by HESKA Corp were from a different company other than the ones used for IDT (i.e. Greer). We chose to use Heska because they offered to make a study specific customized serum test panel based only on the allergens used in our study and because of the specificity of IgE detection using its FcεR1α-based assay.⁷ Ideally, IDT allergens and allergens for *in vitro* testing should be from the same manufacturer, however previous studies compared IDT and serum allergy testing in allergic dogs showed agreement despite lack of standardization with extract manufacturer.⁸

Although IDT is considered to be one of the most important tests to confirm atopy, standardization is still lacking in test procedures. It is not unusual for up to 30% of dogs with a clinical diagnosis of atopy to have a negative IDT.² Based on revised nomenclature these dogs may have canine atopic-like dermatitis in which they have inflammatory and pruritic skin disease but IgE response to environmental or other allergens can not be documented.^{9,10} Many other factors, such as different or too low skin test concentrations or different methods of evaluating skin test reactions among dermatologists, have also been suspected to influence the sensitivity of the test. The histamine concentration 1 : 10 000 (0.1 mg/mL) used in this study was chosen because it appears to show more consistent reactions and a more significantly different diameter than a histamine concentration of 1 : 100 000.^{5,11} Objective evaluation of IDT reactions is considered one of the established evaluation methods and was chosen for this study.³

The determination of optimal testing concentrations for IDT is pivotal for proper use of the test in allergic animals and could increase the sensitivity of IDT. Optimal testing concentrations have been arbitrarily referred to as the allergen's threshold concentration or the highest concentration that does not produce irritant (false-positive) reactions in at least 90% of normal individuals.¹ In addition, many aspects of the test procedure could account for a

negative reaction but one interesting possibility is that the concentrations used are below the threshold concentration, and allergic animals may fail to react, resulting in a false-negative reaction. A more recent study attempted to better define the threshold concentrations by studying the results of using serially higher concentrations in normal dogs.⁵ Based on those results, threshold concentrations could not be determined at the highest concentration tested; therefore, expanding on the highest concentration tested was attempted to determine if threshold concentrations could be established.

Based on the hypothesis that threshold concentrations are those where less than 10% of normal animals have positive reactions at a given concentration, we utilized serial dilutions of allergens to evaluate a concentration where greater than or equal to 10% of the animals tested showed positive reactions. This concentration would then give the concentration where irritant (false-positive) reactions were produced; therefore, suggesting that the optimal testing concentration is equal to or less than the determined concentration.

For only five allergens could the ≥10% ITC be determined, ranging from 2750 to 7000 PNU/mL, and these concentration were higher than the currently recommended testing concentrations of 1000 PNU/mL; for all others it could not be determined and might be at least 8000 PNU/mL, the highest concentration tested.

Concurrently serial dilutions were also evaluated to determine at what concentration 0% of all the dogs tested had positive reactions. An ITC using the 0% cut-off is the concentration where no dogs react at this and all lower concentrations tested. This was performed to evaluate what difference was seen if threshold concentrations were defined using a 0% cut-off instead of a 10% cut-off as finding a 10% ITC could not be established for many allergens. For 12 of the allergens tested we were able to determine the 0% ITC, and all were greater than the currently recommended testing concentration. For six of the allergens no 0% ITC could be determined because at least one dog reacted to each concentration tested. For all other allergens the 0% ITC could not be determined and could be greater than 8000 PNU/mL, the highest concentration tested.

Our findings are similar to previous studies conducted in both cats, where the ITC could not be determined, and horses where the appropriate testing concentration may

be >1000 PNU/mL.^{12,13} In these studies the ITC was evaluated based on a 10% cut-off. Even with a 0% cut-off used in this study it was not possible to determine the ITC for several allergens because they may be higher than the greatest concentration of 8000 PNU/mL tested here. Based on the results of this study it appears difficult to induce an irritant or 'false' positive reaction with even very high test concentrations in clinically healthy dogs. No adverse reactions were observed to high IDT concentrations in any of the study participants. These results suggest that ITCs may vary, also they may be very high for some allergens tested and that higher test concentrations may be used for IDT for the tested allergens without inducing an irritant (false-positive) reaction. However, further studies are needed to evaluate the benefit of higher IDT concentrations and the safety of higher test concentration in atopic dogs.

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Résumé La concentration irritante seuil (ITC) des tests intradermiques (IDT) a été déterminée chez 31 chiens sains cliniquement non-allergiques. Vingt-trois allergènes ont été testés à cinq concentrations différentes entre 1000 et 8000 PNU/mL. Pour différencier les réactions irritantes des hypersensibilités sub-cliniques médiées par les IgE, des tests d'allergies sériques ont été réalisés. Les ITC ont été évalués à partir de la plus faible concentration à laquelle aucun chien (0%) et au moins 10% des chiens (≥10%) réagissaient. Les ITC à la limite de 0% étaient : 1000 PNU/mL (herbe de Johnson), 2000 PNU/mL (Ash, Lamb's Quarter et Bermuda), 3000 PNU/mL (Bahia, Rye, Pig Weed, et Virginia Oak), 4000 PNU/mL (Marsh Elder et Maple), 5000 PNU/mL (Sorrel sheep), et 7000 PNU/mL (Cocklebur et Black Willow). Les ITC pour la camomille, l'érable et le cèdre rouge étaient <1000 PNU/mL. Les ITC pour des limites ≥10% étaient : 2500 PNU/mL (Johnson), 3000 PNU/mL (Box Elder), 5000 PNU/mL (Bahia), 6000 PNU/mL (Pigweed et Marsh Elder), et 8000 PNU/mL (Virginia Oak et Black Willow). Pour tous les autres allergènes, les ITC étaient >8000 PNU/mL et ne pouvaient pas être déterminés. Aucune correspondance significative n'a été trouvée pour le même allergène entre les valeurs positives des IDT et des tests sériques pour chaque chien suggérant ainsi que les réactions dues aux ITC n'étaient probablement pas des réactions sub-cliniques médiées par les IgE. Ces résultats suggèrent que les ITC peuvent varier, être très élevés pour les allergènes testés et que des concentrations plus élevées pourraient être utilisées pour les IDT sans induire de réactions irritantes. Des études supplémentaires sont nécessaires pour évaluer les avantages d'une utilisation de concentrations d'IDT plus élevées chez les chiens atopiques.

Resumen Se determinó la concentración límite inicial (ITC) para la prueba intradermal (IDT) en 31 perros sanos sin signos de alergia. Se probaron 23 alérgenos a cinco concentraciones variables entre 1000–8000 PNU/ml. Para diferenciar las reacciones de irritación de la hipersensibilidad subclínica mediada por IgE se realizaron pruebas serológicas de alergia. Las ITC se determinaron evaluando la menor concentración a la cual ningún perro (0% valor de corte) en la cual al menos 10% de los perros (≥10% valor de corte) reaccionaron. Las ITCs con un valor de corte del 0% fueron: 1000 PNU/ml (sorgo), 2000 PNU/ml (fresno, cenizo y grama), 3000 PNU/ml (bahía, centeno, *Amaranthus sp.* y roble de Virginia), 4000 PNU/ml (*Iva annua* y arce), 5000 PNU/ml (*Rumex acetosella*), y 7000 PNU/ml (arrancamoños y sauce negro). La ITC

para *Eupatorium capillifolium*, arce plateado, y cedro rojo fue <1000 PNU/ml. Las ITCs al valor de corte de $\geq 10\%$ fueron: 2500 PNU/ml (sorgo), 3000 PNU/ml (arce plateado), 5000 PNU/ml (bahía), 6000 PNU/ml (*Amaranthus sp.* y *Iva annua*), y 8000 PNU/ml (roble de Virginia y sauce negro. Para el resto de alérgenos la ITC fue >8000 PNU/ml y no se pudo determinar. No hubo una equivalencia significativa entre valores positivos para el mismo alérgeno entre la prueba intradérmica y la prueba serológica para cada perro, lo cual sugiere que reacciones causadas la ITC determinada tienen poca probabilidad de ser reacciones subclínicas mediadas por IgE. Estos resultados indican que la ITC puede variar, que pueden ser muy elevadas para el alérgeno en cuestión y que las altas concentraciones pueden utilizarse en la prueba intradérmica sin inducir una reacción irritante. Se necesitan más estudios para evaluar el beneficio de más altas concentraciones en la prueba intradérmica en perros atópicos.

Zusammenfassung Bei 31 gesunden, klinisch nicht-allergischen Hunden wurde die „irritierende“ Schwellenwert-Konzentration (ITC) für den Intradermaltest (IDT) bestimmt. Dreiundzwanzig Allergene wurden in fünf unterschiedlichen Konzentrationen von 1000–8000 PNU/mL getestet. Um irritierende Reaktionen von subklinischen IgE-vermittelten Überempfindlichkeiten zu unterscheiden, wurden Serum Allergietests durchgeführt. Die ITCs wurden durch die Bestimmung der niedrigsten Konzentration auf die kein Hund (0% Cut-off) und auf die mindestens 10% der Hunde ($\geq 10\%$ Cut-off) reagierten, ermittelt. Die ITCs beim 0% Cut-off betrugen: 1000 PNU/mL (Wilde Mohrenhirse), 2000 PNU/mL (Esche, Weißer Gänsefuß und Bermudagrass), 3000 PNU/mL (Bahiagrass, Roggen, Amaranth und Virginia-Eiche), 4000 PNU/mL (schwarzer Holunder und Ahorn), 5000 PNU/mL (Kleiner Ampfer) und 7000 PNU/mL (Euphorbia und Schwarz-Weide). Die ITC für die Hundskamille, Eschen-Ahorn und Rote Zeder waren <1000 PNU/mL. Die ITCs beim $\geq 10\%$ igen Cut-off waren: 2500 PNU/mL (Wilde Mohrenhirse), 3000 PNU/mL (Eschen-Ahorn), 5000 PNU/mL (Bahiagrass), 6000 PNU/mL (Amaranth und schwarzer Holunder), und 8000 PNU/mL (Virginia-Eiche und Schwarz-Weide). Für alle anderen Allergene betrug die ITC > 8000 PNU/ml und konnte nicht bestimmt werden. Es wurde keine signifikante Übereinstimmung zwischen den positiven Ergebnissen desselben Allergens im IDT und im Serum Allergietest für die einzelnen Hunde gefunden, was darauf hinweist, dass es sich bei den Reaktionen, die durch die ermittelten ITCs entstanden sind, eher nicht um subklinische IgE-vermittelte Reaktionen handelt. Diese Ergebnisse zeigen, dass die ITCs variieren könnten, dass sie für die getesteten Allergene sehr hoch sein können und dass für die untersuchten Allergene vermutlich höhere Testkonzentrationen für den IDT verwendet werden können ohne eine irritierende Reaktion auszulösen. Weitere Studien sind notwendig, um den Vorteil höherer IDT Konzentrationen bei atopischen Hunden zu eruieren.