

Performance characteristics of a monoclonal antibody cocktail-based ELISA for detection of allergen-specific IgE in dogs and comparison with a high affinity IgE receptor-based ELISA

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Conflict of Interest

Kenneth W. Lee, Karen D. Blankenship, Zachary M. McCurry, and Robert E. Esch are employees at Greer Laboratories Inc. Douglas J. DeBoer and Rosanna Marsella serve as members of Greer's Veterinary Scientific Advisory Board.

Abstract

The purpose of this study was to define the operational and performance characteristics of a commercially available monoclonal antibody based (mac) ELISA for detection of allergen-specific IgE in dogs. The average intra-assay variance over 1 year was 9.7% (range 2.5–62.7%), while the interassay variance averaged 10.8% (range 8.1–13.8%). The average positive control responses observed for grass, weed, tree and mite allergens during each month remained relatively constant; the average monthly variance was 11.6% (range 8.3–19.2%) for grass pollens, 13.3% (range 9.1–20.4%) for weed pollens, 13.3% (range 9.8–18.2%) for tree pollens and 13.6% (range 8.9–18.7%) for mite allergens. The interlaboratory concordance of results for the macELISA was approximately 91%. The interlaboratory concordance of results comparing the macELISA and a high affinity IgE receptor-based ELISA was approximately 92%. The results demonstrate that the macELISA is reproducible and the results are comparable to the high affinity IgE receptor based ELISA within and between laboratories.

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Introduction

Allergic diseases have been demonstrated in humans, dogs, cats, horses, cows and a variety of laboratory animals.^{1,2}

The epidemiology, pathogenesis, clinical signs, diagnosis and treatment of canine atopic dermatitis (AD) have been reviewed by the American College of Veterinary Dermatology Task Force on Canine Atopic Dermatitis (currently the International Task force on Canine Atopic Dermatitis (ITFCAD)).^{3–14} It is likely that the pathogenesis is similar to humans.^{1–5,15–18} The majority of cases appear to be associated with IgE-mediated type I (immediate) hypersensitivity reactions.^{1,3} The diagnosis is based on a suggestive history, typical clinical signs and ruling out differential diagnoses.^{1,6,7} Allergen-specific immunotherapy is a safe and effective therapeutic option for long-term management of canine AD.^{8,9,19}

Allergy testing is used to determine reactive allergens for avoidance and immunotherapy. Two basic types of tests are available: intradermal tests¹⁰ (IDT or 'skin tests') and allergen-specific IgE serology (IgE serum tests).¹¹ The presence and quantity of allergen-specific serum IgE do not, however, necessarily correlate with severity of clinical signs. IDTs, which detect the functional ability of cutaneous mast cells to degranulate upon exposure to allergen extracts, are considered more sensitive but again do not always correlate with clinical signs.^{10,11} The results of these tests should be interpreted in light of the history, seasonality and environmental exposure of the patient.

It has been more than two decades since the functional characteristics of the first commercially available *in vitro* assay for detection of allergen-specific IgE in dogs were described.²⁰ There are now many other commercial assays, all of which rely upon the use of either polyclonal or monoclonal anti-IgE antibodies, or a recombinant human high-affinity IgE epsilon receptor fragment to detect canine IgE.^{21,22} Comparative evaluations have attempted to demonstrate superiority of one method over another.^{11,23–26} Technological advances have improved reagents and procedures,²⁷ making previous comparisons obsolete. Consequently, there is a paucity of useful data comparing commercially available tests, and assessing intra- and interlaboratory consistency among the various veterinary *in vitro* testing services. In evaluating serum-based testing, the Task Force reported that methodology for these tests varies by laboratories, few critical studies have evaluated their performance, and concluded that current interlaboratory standardization and quality control measures are inadequate.¹¹

To address these issues, this study was designed to define the performance characteristics of a commercially available monoclonal antibody cocktail-based ELISA (macELISA) and to compare results from the manufacturer's

reference laboratory (Greer Laboratories, Lenoir, NC, USA) with those observed in one of its European affiliate laboratories (ARTU Biologicals, Lelystad, the Netherlands). The macELISA was further compared to high affinity IgE receptor (FcεR1α; fceELISA) based assay (Heska Corporation, Fort Collins, CO, USA) in a double-blind study.

Materials and methods

Serum samples

All dog sera used throughout the development and characterization of the macELISA were received as individual samples, although positive and negative serum pools prepared from these samples were also used in some experiments. Two positive and one negative control samples derived from single lots were run on each of the allergen panels.

Calibrators

Calibrator solutions of predetermined reactivity in the macELISA were prepared as twofold serial dilutions (calibrators no. 1–4) of a sera pool highly reactive to most pollen allergens. An additional calibrator (no. 5) was prepared from sera shown to be negative to pollen allergens in the macELISA. The reactivity of these calibrators to a mixture of grass pollen allergens was determined in each assay run. The calibrator solutions were derived from two lots.

Allergen-coated microwells in the macELISA

All allergen extracts used in this study were acquired from Greer Laboratories, Inc. and were prepared following a standard proprietary extraction protocol. Extracts were 1 : 10 or 1 : 20 w/v products, or they were standardized to relative potency,^{28,29} and the optimal well coating concentration was determined by dilution titration. All extracts were received as either aqueous or glycerinated products, and with exception of dilution in 50 mM carbonate buffer (pH 9.6), were used without further processing. Immulon 4 HBX Removawell Assemblies (Thermo Labsystems, Franklin, MA, USA) were used. Each allergen extract was diluted to a predetermined optimum coating concentration and 100 µL of each diluted extract was added to the microwells. Following overnight (12–16 h) incubation at 4–8 °C, each well was washed once with PBS-T (50 mM phosphate-buffered saline containing 0.05% Tween 20), and blocked with 400 µL of 1% monoethanolamine pH 7.5 for 1–2 h at room temperature. The wells were then washed twice with PBS-T, air dried for 1–2 h, packaged in zip-lock plastic bags and stored at 4–8 °C until used. Multiple lots of allergen-coated wells were used throughout the study: lot no. 1 in January and February; lot no. 2 March to April; lot no. 3 early June to mid-September; and lot no. 4 during the remainder of the year.

Canine IgE detection reagents

Monoclonal anti-IgE antibodies (IgG1κ or IgG2Aκ) were isolated from hybridoma tissue cultures using Protein A affinity chromatographic columns. The specific IgE reactivity of each isolate was confirmed by strong positive immediate type reactions following intradermal injection into dog skin. In addition no specific reactivity was demonstrated in ELISAs specifically designed to detect dog IgM, IgG or IgA.

Biotinylation of monoclonal anti-IgE was accomplished using EZ-Link Sulfo-NHS-LC-Biotin ester (Pierce Chemical Co., Rockford, IL, USA) following the manufacturer's recommended procedure. Briefly, individual monoclonal anti-IgE solutions were dialysed against 50 mM carbonate buffer pH 8.5. A 6 mM solution of biotin ester in bicarbonate buffer was added to each monoclonal solution to yield a molar ratio mixture of 12 : 1 (biotin:monoclonal). Following incubation at room temperature for 2 h with constant agitation, excess biotin ester was removed by dialysis against tris-buffered saline. The protein recovered from each reaction mixture was estimated by determining the optical density at 280 nm, assuming an extinction coefficient of 1.4 for each monoclonal IgG. A sufficient volume of glycerol was added to each monoclonal-biotin conjugate to yield a 50% solution before storage at –20 °C.

The IgE specificity of the monoclonal anti-IgE was confirmed by documenting the heat lability (56 °C) of reactive serum components. A mixture of three monoclonal anticanine IgE antibody preparations was optimized for use in the IgE-specific ELISA. The antibodies were selected on the basis of their high specific reactivity to dog IgE and low background responses when used at high concentration. The protein concentration of the stock mixture was adjusted to 100 µg mL^{–1}, with the protein content of each monoclonal proportionate to its reactivity when evaluated alone.

macELISA (Aller-g-Complete™; Greer Laboratories)

The macELISA for allergen-specific IgE in dog serum was optimized by individually varying the reagent concentrations or incubation times, while maintaining all other conditions constant. Briefly, serum samples were diluted 1 : 6 in diluent buffer (PBS-T containing 0.2 mM MgCl₂ and 10 mg mL^{–1} fish gelatin). One hundred microlitres of the diluted samples was added to coated microwells and incubated overnight (12–16 h) at 4–8 °C. The wells were washed once with PBS-T, and 100 µL of biotinylated monoclonal anti-IgE-biotin mixture in diluent buffer was added to each well. After 2 h incubation at room temperature (22 °C), the wells were washed twice with PBS-T, and 100 µL of streptavidin-alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA, USA) in diluent buffer was added to each well before incubation for 1 h at room temperature. Following a final washing (four cycles with PBS-T), 100 µL of pNPP substrate (Moss, Inc., Pasadena, MD, USA) was added to each well. The enzymatic reaction was stopped after 1 h with 50 µL of 20 mM cysteine to each well. The optical density (OD) of each well was then determined at 405 nm.

All results were expressed in ELISA allergy units (EAU), which were calculated as the OD in milli absorbance units corrected for background (sample OD – background OD × 1000) normalized to a four point calibration curve. The cut-off level (99% confidence interval) for this ELISA has been established at 150 EAU by evaluating reactivity in sera from specific pathogen-free animals and in heated sera. Serum and plasma have been shown to be of equal utility in this ELISA, and no adverse affects have been documented using haemolysed or lipaemic sera. During development of the assay, the average intra-assay coefficient of variation (CV) was 6.7% (range 4.0–8.9%) and the average interassay CV was 8.7% (range 7.0–10.6%).

Interlaboratory evaluation of the macELISA

The sera used to compare the interlaboratory variability of the macELISA included samples from ARTU Biologicals, a European affiliate laboratory. On two separate occasions before evaluation, the samples were split with one aliquant shipped to Greer's reference laboratory for testing while the other was evaluated on a panel of 21 allergens in ARTU's laboratory. The results remained confidential to the evaluating laboratory until all results were collected, and were then simultaneously exchanged. Once the laboratory evaluations were completed, all results were forwarded to the USA facility for analysis.

Interlaboratory evaluation of the macELISA and the fceELISA

The samples used to compare macELISA and fceELISA were received from Greer's reference laboratory as individual samples, and each was supplied with its macELISA-defined allergen reactivity profile. A negative serum pool was prepared from samples that were nonreactive to any of the allergens tested. Positive sera pools of varying allergen reactivity that ranged from specific mite reactivity to those that exhibited multiple reactions to pollen allergens were prepared. Portions of two positive pools that exhibited high and intermediate levels of pollen reactivity were heated to 56 °C for 4 h and used as negative control samples. A heated negative serum pool was included as a control for non-specific reactions. Four 2 mL aliquants from 18 different positive and negative serum pools were prepared. The identity and reactivity of the pools remained confidential throughout the study. Replicate samples were submitted to Greer's reference laboratory and to Heska's reference laboratory (Loveland, CO, USA). No personnel undertaking the assays at either commercial laboratory were aware of the experimental design conditions or the replicate

nature of the samples. Each laboratory was requested to evaluate the samples on a panel of allergens representative of the north-east USA region routinely offered and all results were presented in EAU.

Results

Canine IgE detection reagents

The results presented in Figure 1 are representative of the reactivity detectable with each of the biotinylated anti-IgE antibodies and mixtures used with serum from a dog specifically sensitized to *Dermatophagoides farinae*. The IgE character of the reaction was confirmed by heat inactivation of the serum reactive component. The signal generated using antibody 1 was approximately twice that generated using either antibody 2 or 3. Mixing the antibodies in equal concentration resulted in an additive signal. The biotinylated antibodies 1, 2 and 3 were therefore mixed in a 1 : 2 : 2 ratio in the macELISA.

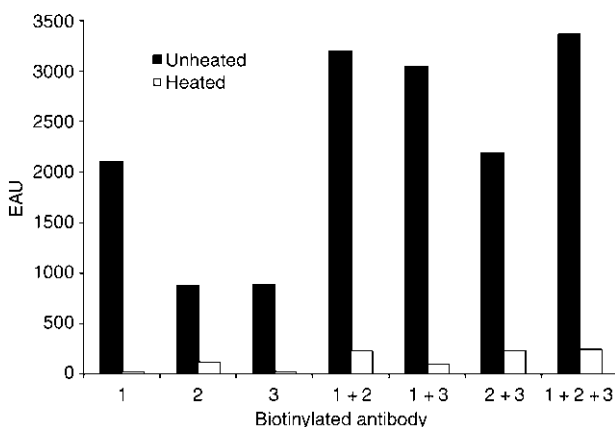


Figure 1. IgE reactivity specific to *Dermatophagoides farinae* detected in unheated and heated serum samples by individual biotinylated antibodies and admixtures of the individual solutions. Equal concentrations of biotinylated antibodies were included in the mixtures and each was adjusted to contain 100 ng mL⁻¹ in the working solution. EAU, ELISA allergy units.

Reproducibility of macELISA

One hundred and ninety-four assay runs were completed during the study, with an average of 15 calibrator evaluations per day (range 8–28). The average intra-assay CV was 9.7% (range 2.5% and 62.7%). The overall and monthly interassay CVs are presented in Table 1. The interassay CV among reactive calibrators (no. 1–4) remained relatively constant (average 10.8%; range 8.1–13.8%). Variability was higher with negative calibrator no. 5 (average 15.6%; range 12.5–21.1%), while the greatest variability was evident with the background ODs (average 20.1; range 12.6–30.7%).

The average responses with the negative control sera (Table 2) provided data for the cut-off level for determining positive responses in the assay. The average negative response observed for grasses was 37 ± 27 EAU, for weeds 22 ± 23 EAU, for trees 33 ± 46 EAU and for environmental allergens 44 ± 52 EAU. The overall average observed negative response was 34 ± 37 EAU.

The results presented in Figure 2 are the average reactive control responses observed for representative grass, weed, tree and mite allergens during the study. The reproducibility of these responses is representative of the responses observed with all allergens tested. The within lot month to month variability remained relatively constant – the average monthly CV was 11.6% (range 8.3–19.2%) for grass pollen allergens, 13.3% (range 9.1–20.4%) for weed pollen allergens, 13.3% (range 9.8–18.2%) for tree pollen allergens and 13.6% (range 8.9–18.7%) for mite allergens.

Interlaboratory reproducibility of the macELISA

The results presented in Figure 3 show that the interlaboratory concordance of results was at least 91%. All the discordant results were at or very near the cut-off level of 150 EAU. This apparent assay discordance is a function of attempting to establish an absolute cut-off value within a dynamic range of responses, and is also observed with varying cut-offs of 150, 250, 500 or 1000 EAU. The lack of concordance remained relatively constant between 6 and 9%, which is consistent with the single laboratory interassay variability (Table 1).

Table 1. Interassay reproducibility for canine Aller-g-complete® ELISA performed in Greer's reference laboratory (Lenoir, NC, USA)

Month	Calibrator lot no.	N	Calibrator† % CV					Background*
			No. 1	No. 2	No. 3	No. 4	No. 5	
January-07	1003-01	220	11.1	11.7	12.6	13.0	17.9	17.7
February-07	1003-01	222	10.8	9.8	10.2	9.6	12.5	22.4
March-07	1003-01	256	10.3	11.3	11.0	10.5	17.8	17.0
April-07	1003-01	262	10.3	8.9	8.9	8.8	15.0	27.4
May-07	1003-01	248	13.1	13.3	13.8	11.7	21.1	20.1
June-07	1003-01	220	10.5	10.3	10.3	9.8	12.7	19.2
July-07	1003-01	250	13.3	12.5	12.4	11.3	16.4	18.2
August-07	1003-01	192	10.1	10.8	10.3	9.6	13.3	14.8
September-07	1003-14	288	10.6	10.4	9.9	9.9	12.6	21.7
October-07	1003-14	294	11.4	12.4	12.3	10.3	17.1	12.6
November-07	1003-14	236	10.3	10.0	9.9	9.8	17.6	30.7
December-07	1003-14	224	10.0	10.0	9.8	8.1	13.6	19.9
Jan–Aug 2007	1003-01	1870	11.6	11.3	11.3	10.4	16.5	21.2
Sept–Dec 2007	1003-14	1042	11.0	11.5	11.6	10.4	15.9	22.8
Overall	1003-01 & 1003-14	2912	13.1	12.2	14.4	12.2	17.0	21.8

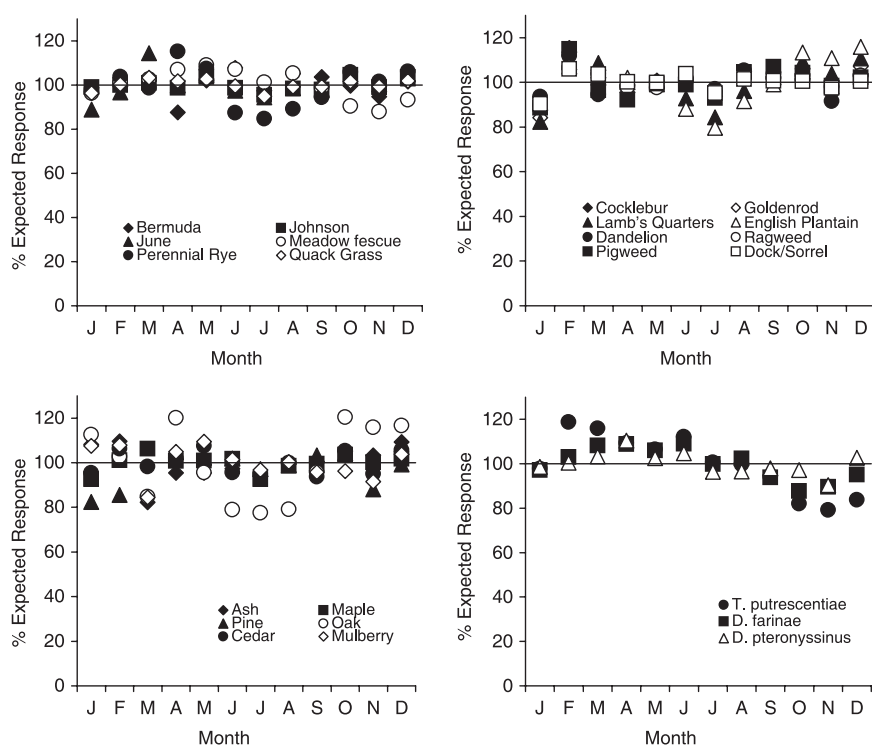
*Responses observed with diluent in place of serum sample.

†The reference calibrators were prepared from a stabilized canine serum pool that contains a high level of IgE-specific for grass pollens. Calibrators 1–4 are prepared as a twofold serial dilution of the serum pool, and calibrator 5 is prepared from a known negative serum pool.

Table 2. Verification of cut-off values for determining positive responses using the Aller-g-complete® ELISA

Allergens	N	Average negative response $\pm$ SD*	Positive response cut-off level confidence		
			68%	95%	99%
Grasses	1 862	37 $\pm$ 27	64	91	118
Weeds	2 724	22 $\pm$ 23	45	69	92
Trees	4 079	33 $\pm$ 46	79	124	170
Environmental	1 663	44 $\pm$ 52	96	147	199
Combined	10 328	34 $\pm$ 37	71	108	145

*Average $\pm$ standard deviation (SD) of background corrected negative responses expressed in milli absorbance units (EA units) measured at 405 nm.

**Figure 2.** Average monthly response of positive control sera evident in the macELISA (Greer Aller-g-complete®) ELISA during 2007.

Interlaboratory evaluation of the macELISA and the fceELISA

The summary results of the blinded study which compared the Greer macELISA and the Heska fceELISA are presented in Figure 4. A total of 18 samples were evaluated in duplicate, giving a total of 558 replicate observations for the 31 allergens common to both panels (6 grasses, 6 weeds, 12 trees, 3 mites and 4 fungi). The concordance for grass pollen allergens ($n = 108$) was 93.5%, for weeds ($n = 108$) 93.5%, for trees ($n = 216$) 89.4% and for the combined pollens ($n = 432$) 91.4%. The concordance with mite allergens ($n = 54$) was 98.2% and for the various fungi ($n = 72$) it was 90.3%. The overall concordance with the results of the two ELISA's was 92%, which closely approximates interlaboratory comparisons of the macELISA (Figure 2). The majority of discordant observations occurred around the assay cut-off.

Heating of reactive samples resulted in at least a 90% reduction in reactivity in both the macELISA and the fceELISA (Table 3). The comparative dose-response of macELISA and fceELISA is presented in Figure 5. The profile for each test follows a second order polynomial function (i.e. quadratic), which is consistent with expected reactivity profile for assays of this type.³⁰

Discussion

The specificity of the monoclonal antibodies used in this study for canine IgE was confirmed by strong positive immediate type reactions following intradermal injection into dog skin and by lack of specific reactivity to dog IgM, IgG or IgA. The heat lability of the reactive serum component provided further confirmation that the biotinylated antibodies were specific for IgE. The additive effect evident with mixtures of biotinylated antibodies suggested that the individual antibodies detect distinct IgE epitopes. Cross-inhibition ELISAs using nonbiotinylated antibodies as competitors further demonstrated that the specific epitope reactivity for each anti-IgE antibody did not overlap (data not presented). The heat susceptibility of these epitopes confirmed they reside in the Fc portion of the IgE molecule, and are probably at or very near to the Fc receptor binding moiety.

The CV of the positive calibrator responses was relatively consistent and remained constant from month to month. The variability of background and negative sera responses, in contrast, was substantially greater. The increased variability at the lower end of the dose-response curve probably occurs because even minute variability will account

		Cutoff = 150 EA Units		Cutoff = 250 EA Units	
		Greer		Greer	
		Positive	Negative	Positive	Negative
ARTU	Positive	333	55	268	40
	Negative	62	831	56	917
Total Sample Pairs		1281		1281	
Number Concordant		1164		1185	
% Concordance		90.9		92.5	

		Cutoff = 500 EA Units		Cutoff = 1000 EA Units	
		Greer		Greer	
		Positive	Negative	Positive	Negative
ARTU	Positive	188	39	141	26
	Negative	55	999	52	1062
Total Sample Pairs		1281		1281	
Number Concordant		1187		1203	
% Concordance		92.7		93.9	

Figure 3. Interlaboratory concordance of results from the macELISA (Greer Allerg-complete®) as a function of cut-off level.

		Combined Allergens	
		Greer	
		Positive	Negative
Heska	Positive	217	19
	Negative	26	296
Total Sample Pairs		558	
Number Concordant		513	
% Concordance		91.9	

Figure 4. Concordance of results between the macELISA and fceELISA.

for a substantial portion of the observed response. The increased variability with negative responses emphasizes the need to establish an appropriate and robust cut-off for each assay. For the macELISA, a cut-off of 150 EAU establishes 99% confidence for positive responses.

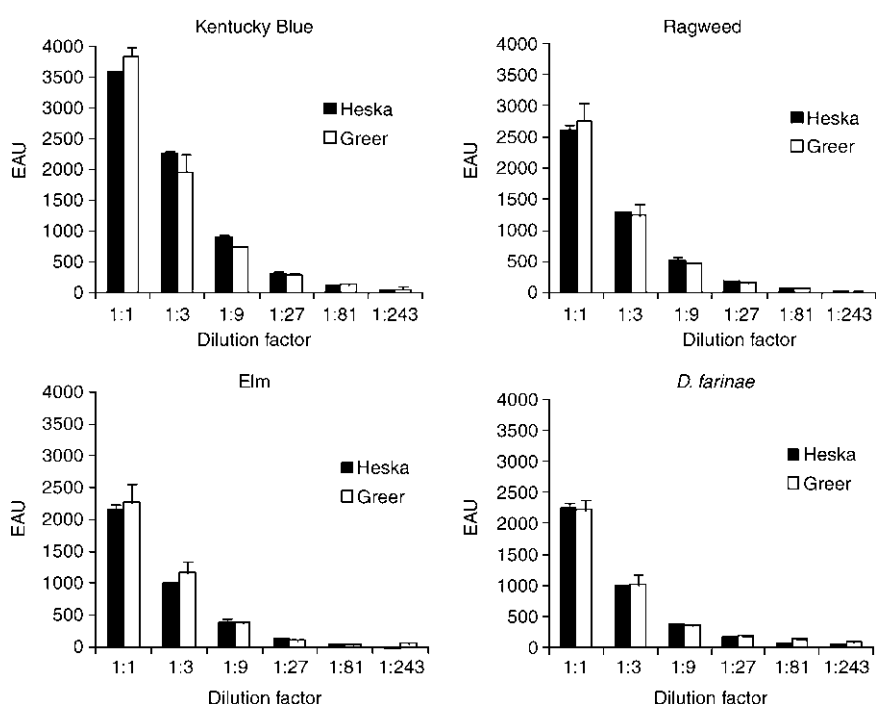
One of the pitfalls of establishing a universal cut-off for an assay which provides responses across a dynamic

range is that the cut-off is generally applied to all allergens included in the assay. This study found that variability among negative responses for grasses and weeds was relatively constant and well below the general assay cut-off level. However, greater variability among trees and other environmental allergens places the cut-off above the overall general cut-off established for the assay. The number of species and diversity of allergens encompassed by this group of allergens probably contribute to this increased variability. It might be more appropriate therefore to establish cut-off levels for each allergen evaluated in an assay. For example, in this study, responses to grasses and weeds that exceed 100 EAU might be considered reactive (99% confidence) while tree and other environmental allergen responses might be considered positive (99% confidence) only when the responses exceed 170 and 200 EAU, respectively. It should also be noted that these ELISA results are not quantitative but semiquantitative at best,³⁰ and that the results are usually interpreted as qualitative only.

The use of different lots of allergen coated wells contributed to the observed variability throughout the year. For example, for the lot used in June to August the responses observed with perennial rye grass were less than the annual average while responses for meadow fescue were slightly greater than the annual average. These trends are almost exactly reversed with the lot of coated wells used in October to December. A similar lot-to-lot

Table 3. Effect of heating serum on the response evident in a monoclonal-based ELISA and comparison with response evident in an FcεR1α-based ELISA

Allergen	Positive control (EA)				Negative control (EA)			
	Unheated		Heated		Unheated		Heated	
	Greer	Heska	Greer	Heska	Greer	Heska	Greer	Heska
Bermuda	3928	3385	343	61	51	18	160	26
Johnson	4114	3363	239	2	31	11	126	0
June	3796	3592	175	49	23	18	71	16
Meadow fescue	3484	3563	131	170	24	29	39	37
Perennial Rye	2818	3490	131	61	20	16	47	22
Timothy	3261	3609	151	71	32	17	89	22
Dock/Sorrel	3089	3563	249	56	29	16	67	20
Cocklebur	1816	1842	125	64	5	15	63	23
English Plantain	2997	3281	103	51	18	19	61	18
Lamb's Quarters	1854	2072	146	77	33	11	72	20
Pigweed	1365	2281	205	62	45	8	155	15
Ragweed	2730	2623	243	40	19	18	136	23
Ash	2082	1647	130	18	11	17	47	0
Bayberry	1742	2752	176	0	18	2	64	0
Birch	1549	666	131	34	13	5	102	10
Cedar	1363	2245	265	33	52	5	176	10
Cottonwood	1600	2267	108	43	12	5	74	15
Elm	2251	2172	119	16	5	0	103	0
Hickory	2855	2747	125	23	21	1	61	18
Maple	3589	3162	158	38	21	4	60	29
Mulberry	1527	2137	101	40	10	6	58	18
Sycamore	2483	3782	140	32	21	7	107	17
<i>D. farinae</i>	2200	2243	178	32	92	59	73	16

**Figure 5.** Comparison of dose-response characteristics of the macELISA (Greer Aller-g-complete® ELISA) and the fceELISA (Heska Allercept™ ELISA).

variability was also obvious for other allergens. Nevertheless, the results observed with the various lots are within the acceptable variance limits ($\pm 20\%$) established for our laboratory.

It is emphasized that detection of allergen-specific IgE by any modality should not be interpreted as being diagnostic. The initial diagnosis of allergy is made clinically with the fulfilment of a combination of criteria that are strongly associated with the disease,^{6,11} and the results

observed with ELISA and/or IDT can only be supportive of this definitive diagnosis. The true utility of an allergen-specific IgE assay lies in the selection of allergens for immunotherapy or allergen avoidance. Consequently, the relatively low and consistent interassay, and intra- and interlaboratory variation of the macELISA supports its reliability and robustness. The results from the macELISA are also directly comparable to those from the fceELISA. The apparent residual reactivity observed in the heated

positive serum with macELISA compared to fceELISA might be a result of incomplete inactivation of the various IgE epitopes detected by the anti-IgE cocktail compared to the single binding site specificity of the fceELISA. Despite this, magnitudes of response and the dose–response profile following dilution of reactivity in unheated sera were similar in both assays. This indicates that differences in the IgE detection system, allergen coated wells, buffers and procedures do not necessarily affect assay results. It is possible that the minor differences observed reflect the allergens used to coat the wells and not an overall discrepancy between the assay methodologies. A consistency monitoring programme of manufactured allergen extracts should therefore be established.

This report demonstrates that it is possible to establish and monitor intra- and interlaboratory standardization. This will help to ensure the uniformity, consistency and reproducibility of results. A proficiency monitoring programme among veterinary laboratories similar to peer comparison and monitoring programmes for human laboratories should be established. Such a programme should allow laboratories to evaluate their performance and improve the accuracy of the results they provide, giving the end-user more confidence. This study does not, however, necessarily provide robust data on the clinical usefulness of either test for canine AD. This would require comparison of these tests and IDTs in specific groups of defined atopic dogs, SPF dogs, specifically sensitized laboratory dogs and healthy dogs. Full confirmation would also require comparison of the responses to ASIT and allergen avoidance.

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Résumé Le but de cet étude était de définir les caractéristiques opérationnelles et la performance d'un test ELISA disponible commercialement à anticorps monoclonal (mac) pour la détection d'IgE spécifiques d'allergènes chez le chien. La variance intratest sur un an était de 9.7% (extrêmes 2.5–62.7%), alors que la variance intertest était de 10.8% (extrêmes 8.1–13.8%). Les réponses positives contrôles moyennes observées pour les allergènes d'herbacées, de graminées, d'arbres et d'acariens pendant chaque mois sont restées relativement constantes; la variance moyenne était de 11.6% (8.3–19.2%) pour les pollens d'herbacées, 13.3% (9.1–20.4%) pour les pollens de graminées, 13.3% (9.8–18.2%) pour les pollens d'arbres et 13.6% (8.9–18.7%) pour les pollens d'acariens. La concordance interlaboratoire des résultats du test macELISA était d'approximativement 91%. La concordance interlaboratoire des résultats en comparant le test macELISA et un ELISA réalisé avec le récepteur à haute affinité des IgE était approximativement de 92%. Ces résultats démontrent que le macELISA est reproductible et que ses résultats sont comparables avec le test utilisant le récepteur de haute affinité pour les IgE entre et à l'intérieur des laboratoires.

Resumen El propósito de este estudio fue definir las características operacionales y de funcionamiento de un método comercial ELISA de anticuerpos monoclonales (mac) para la detección de IgE específica de alérgenos en perros. La varianza media interna a la prueba durante un año fue del 9.7% (rango 2.5–62.7%), mientras que la varianza media entre pruebas promedió 10.8% (rango 8.1–13.8%). Las respuestas medias positivas del control para alérgenos del césped, malas hierbas, árboles y ácaros durante cada mes permanecieron relativamente constantes; la varianza media mensual fue 11.6% (rango 8.3–19.2%) para polen del césped, 13.3% (rango 9.1–20.4%) para polen de malas hierbas, 13.3% (rango 9.8–18.2%) para polen de árboles y 13.6% (rango 8.9–18.7%) para alérgenos de ácaros. La concordancia de resultados entre laboratorios para el mac ELISA fue aproximadamente del 91%. La concordancia entre laboratorios comparando resultados del macELISA y del ELISA basado en receptor IgE de alta afinidad fue aproximadamente de un 92%. Estos resultados demuestran que el macELISA es reproducible y que los resultados son comparables con el ELISA de receptor de IgE de alta afinidad en un mismo laboratorio y entre diferentes laboratorios.

Zusammenfassung Das Ziel dieser Studie war es, die Betriebs- und Leistungscharakteristika eines kommerziell erhältlichen ELISAs, welcher zur Bestimmung von Allergen-spezifischem IgE bei Hunden auf monoklonalen Antikörpern (mac) basiert, zu definieren. Die durchschnittliche Intra-Assay Varianz über den Zeitraum eines Jahres betrug 9.7% (Breite von 2.5–62.7%), während die Inter-Assay Varianz im Durchschnitt 10.8% betrug (Breite von 8.1–13.8%). Die durchschnittliche positive Reaktion, die auf Gras-, Unkraut-, Baum- und Milbenallergene in den einzelnen Monaten gesehen wurde, blieb relativ konstant; die durchschnittliche monatliche Varianz betrug 11.6% (Breite von 8.3–19.2%) für Graspollen, 13.3% (Breite von 9.1–20.4%) für Unkrautpollen, 13.3% (Breite von 9.8–18.2%) für Baumpollen und 13.6% (Breite von 8.9–18.7%) für Milbenallergene. Die Inter-Labor Übereinstimmung der Ergebnisse des macELISAs betrug ungefähr 91%. Die Inter-Labor Übereinstimmung der Ergebnisse des macELISAs im Vergleich mit einem ELISA basierend auf einem Hochaffinitätsrezeptor für IgE betrug etwa 92%. Die Ergebnisse demonstrieren, dass der macELISA reproduzierbar ist und die Ergebnisse mit dem auf einem Hochaffinitätsrezeptor für IgE basierenden ELISA innerhalb und zwischen den Labors vergleichbar ist.