

## Agreement between allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories in the USA

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**Background** – Canine allergen-specific IgE assays in the USA are not subjected to an independent laboratory reliability monitoring programme.

**Hypothesis/Objectives** – The aim of this study was to evaluate the agreement of diagnostic results and treatment recommendations of four serum IgE assays commercially available in the USA.

**Methods** – Replicate serum samples from 10 atopic dogs were submitted to each of four laboratories for allergen-specific IgE assays (ACTT<sup>®</sup>, VARL Liquid Gold, ALLERCEPT<sup>®</sup> and Greer<sup>®</sup> Aller-g-complete<sup>®</sup>). The interlaboratory agreement of standard, regional panels and ensuing treatment recommendations were analysed with the kappa statistic ( $\kappa$ ) to account for agreement that might occur merely by chance. Six comparisons of pairs of laboratories and overall agreement among laboratories were analysed for ungrouped allergens (as tested) and also with allergens grouped according to reported cross-reactivity and taxonomy.

**Results** – The overall chance-corrected agreement of the positive/negative test results for ungrouped and grouped allergens was slight ( $\kappa = 0.14$  and  $0.13$ , respectively). Subset analysis of the laboratory pair with the highest level of diagnostic agreement ( $\kappa = 0.36$ ) found slight agreement ( $\kappa = 0.13$ ) for ungrouped plants and fungi, but substantial agreement ( $\kappa = 0.71$ ) for ungrouped mites. The overall agreement of the treatment recommendations was slight ( $\kappa = 0.11$ ). Altogether, 85.1% of ungrouped allergen treatment recommendations were unique to one laboratory or another.

**Conclusions and clinical importance** – Our study indicated that the choice of IgE assay may have a major influence on the positive/negative results and ensuing treatment recommendations.

### Introduction

Allergen-specific immunotherapy (ASIT) is frequently prescribed to aid in the management of canine atopic dermatitis (AD).<sup>1</sup> The formulation of the allergenic extract is customized for each dog based on allergen test results, history and aerobiology.<sup>2</sup> An intradermal test (IDT), serum allergen test (SAT) or both are performed to demonstrate allergen-specific immunoglobulin type E (IgE) directed against a panel of plant, fungi, mite, insect and epidermal antigens deemed to be important in the geographical region.<sup>3</sup> An IDT is usually performed by a clinician using a panel of antigens that they have customized for their location. Within the same geographical region, these panels vary substantially, as do testing methodologies employed by different clinicians.<sup>4</sup> *In vitro* IgE enzyme-linked immunosorbent assays (ELISAs) are offered by at least six commercial laboratories in the

USA, operating without independent oversight of their quality control.<sup>5</sup> Each laboratory divides the USA into aerobiological zones and offers allergen-specific IgE tests for a standard panel of antigens for each zone. These laboratories differ in the number and identity of the antigens tested on standardized panels for a given geographical region. An antigen may be included as a mixture of phylogenetically related species by one laboratory, as individual species by a second laboratory, or not at all by a third.

Allergen-specific IgE levels are interpreted as elevated ('positive') if the optical density measured in the assay is above a cut-off level established by the laboratory.<sup>6</sup> 'Positive' allergens are candidates for inclusion in ASIT extract mixtures. Some of the antigens to which elevated allergen-specific IgE levels are reported may be excluded from the ASIT prescription if they are judged to have caused a false-positive reaction, are cross-reactive with other antigens selected for inclusion, exceed the desired maximal number or are otherwise deemed to be clinically irrelevant based on the patient's history.<sup>7</sup> Commercial SAT laboratories offer technical assistance to support veterinarians in formulating ASIT prescriptions

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based on these factors. Overall, the ASIT prescription for an atopic dog is influenced by the following factors: (i) the selection of allergens to be tested; (ii) the intralaboratory and interlaboratory reliability of the testing; and (iii) post-test interpretation and formulation. Each of these variables may affect the reproducibility of ASIT prescriptions for a given dog.

Despite the potential for variability, the factors that may influence the composition of an ASIT prescription have received relatively little attention, and a variety of allergen-selection methods may be evaluated together in a single ASIT study.<sup>4,8</sup> A rigorous evaluation of the reliability of IDT has not been published. Unpublished studies have reported fair agreement of three investigators interpreting IDT reactions.<sup>9</sup> There is often poor agreement between results of IDT and SAT performed concurrently on the same dog.<sup>6,7,10,11</sup>

Early studies demonstrated poor reliability of SAT; however, the testing methodologies may have changed since those studies were carried out.<sup>5,7,12,13</sup> Subsequently, Thom *et al.*<sup>14</sup> evaluated the reliability of three independent European allergen-specific IgE testing laboratories each using an Fcε receptor ELISA methodology.<sup>14</sup> The authors reported a 3% intralaboratory discrepancy rate and a 9% interlaboratory discrepancy rate for the three laboratories, in terms of all positive and negative reactions. In another report, SAT results were compared between two laboratories that employ the same monoclonal antibody cocktail (mac) ELISA methodology and between one reference laboratory using macELISA methodology and another using the aforementioned Fcε receptor ELISA methodology.<sup>15</sup> Serum was pooled from samples of known macELISA reactivity, and duplicate aliquots were submitted for each of the comparisons. A follow-up to this study reported on the performance characteristics of six laboratories using the macELISA.<sup>16</sup> Agreement of positive and negative reactions was presented as the percentage concordance, which exceeded 90% for interlaboratory comparisons.<sup>14–16</sup> An analysis of the degree to which these concordance rates differed from the agreement that would be expected by chance alone was not presented in these reports. The chance-corrected agreement is important, because tests that are usually negative can have a high intra- or interlaboratory unadjusted agreement rate but a low chance-corrected agreement rate.

The objective of the present study was to evaluate the agreement (unadjusted and chance corrected) of four commercial laboratories offering SAT in terms of the following factors: (i) diagnosis of positive or negative allergen-specific IgE reactivity; and (ii) ASIT treatment recommendations for dogs with atopic dermatitis.

## Materials and methods

### Animals

Client-owned dogs diagnosed with atopic dermatitis were eligible for enrolment in the prospective study. The diagnosis was established by confirming that each dog fulfilled at least five of eight clinical features of canine AD, as described by Favrot *et al.*<sup>17</sup> Differential diagnoses were ruled out as described by DeBoer and Hillier.<sup>18</sup> Dogs

weighing <10 kg were excluded. A sample size of 10 dogs was set as the enrolment goal.

### Sample submission

Approximately 20 mL of blood was collected from each dog with owner consent. Serum from each dog was divided into four aliquots within 30 min, with each aliquot submitted to one of four commercial laboratories for allergen-specific IgE assays [ACTT<sup>®</sup> Bio-medical Services, Inc., Austin, TX, USA (Lab A); VARL Liquid Gold Veterinary Allergy Reference Laboratory, Pasadena, CA, USA (Lab B); ALLERCEPT<sup>®</sup> Heska<sup>®</sup>, Inc., Loveland, CO, USA (Lab C); and Greer<sup>®</sup> Aller-g-complete<sup>®</sup> IDEXX Laboratories, Inc., Westbrook, ME, USA (Lab D)]. Although each of the four assays is based on ELISA techniques, they differ from one another.<sup>15,19–21</sup> To bind canine IgE in their assays, Labs A, B, C and D use polyclonal goat anti-IgE antibodies, a monoclonal rabbit anti-dog αIgE antibody cocktail, a biotinylated recombinant extracellular domain of human FcεRIα receptor and a monoclonal anti-dog IgE cocktail, respectively. The signal molecules used in the assays by Labs A, B, C and D are enzyme conjugate (type unpublished), horseradish peroxidase, streptavidin–horseradish peroxidase and streptavidin–alkaline phosphatase, respectively.

Handling and storage of all samples was identical for each dog. Laboratory-supplied patient history forms were filled out in detail and submitted with the samples. These provided information about the dogs' seasonality of signs and their living conditions that might be taken into account by laboratory consultants when formulating ASIT. Serum samples were refrigerated for no longer than 72 h, and then shipped directly to the laboratories using laboratory-provided vials and mailers. The laboratories were left unaware of the occurrence of the study in order to simulate the day-to-day level of quality control that could be expected by primary care veterinarians. Standard, canine regional allergen-specific IgE panels were requested based upon the dogs' geographical location (southern Texas, USA). Regional panels included 61–63, 40, 48 and 48 allergens or allergen mixtures for Labs A, B, C and D, respectively. A total of 110 individual allergens or allergen mixtures were represented in the four panels (Tables 1 and S1 in Supplementary material). In some cases, there was variation in the spectrum of antigens evaluated by a single laboratory during the course of the study. The antigens evaluated by each laboratory are shown in Table S1 in Supplementary material.

### Interpretation of semi-quantitative scores

Semi-quantitative allergen-specific IgE levels reported by the laboratories were interpreted according to each laboratory's ELISA absorbance unit (EAU) guidelines, when available. Detection of potentially significant allergen-specific IgE levels (interpreted as 'positive') was defined as greater than or equal to 175, 150 and 80 EAU for Labs A, C and D, respectively, in accordance with guidelines provided with each laboratory report. The results from Lab B were interpreted as positive for values greater than or equal to two on a proprietary, six-point ordinal scale (0–5), as previously reported.<sup>13</sup> Levels of allergen-specific IgE below these cut-off values were defined as 'negative' for the antigen.

### Interassay agreement analysis

The agreement of positive and negative findings was analysed in two ways. First, antigens were evaluated separately (ungrouped), not attempting to correct for potentially insignificant differences between assays (e.g. testing a dust mite antigen mixture versus two individual mite species). In order to examine the extent to which subtle differences in regional panels affected our results, a second analysis was performed after grouping individual allergens that are of the same genus or are reported to be cross-reactive or co-sensitizing in dogs or humans (Tables 1 and S1 in Supplementary material).<sup>7,22,23</sup> In this analysis, a positive reaction to any of the grouped components was interpreted as the group being positive; for example, a positive reaction to either one of two tested dust mites by one laboratory and to a mixture of the two mites by another laboratory were treated as displaying agreement in the grouped analysis.

**Table 1.** Complete spectrum of antigens evaluated on standard, canine, southern Texas regional panels by four laboratories with allergen-specific IgE assays

<b>Trees</b>
Alder mix <sup>a</sup> , Arizona ash <sup>b</sup> , white ash <sup>b</sup> , ash mix <sup>b</sup> , bald cypress <sup>c</sup> , bayberry, American beech <sup>d</sup> , birch mix <sup>a</sup> , box elder <sup>e</sup> , box elder/maple mix <sup>e</sup> , California pepper tree, red cedar <sup>c</sup> , cedar/juniper mix <sup>c</sup> , mountain cedar <sup>c</sup> , cottonwood/poplar mix <sup>f</sup> , American elm <sup>g</sup> , elm mix <sup>g</sup> , eucalyptus, hazelnut <sup>g</sup> , maple <sup>e</sup> , maple mix <sup>e</sup> , <i>Melaleuca</i> sp., mesquite, red mulberry <sup>h</sup> , white mulberry <sup>h</sup> , Virginia live oak <sup>d</sup> , oak mix <sup>d</sup> , olive <sup>b</sup> , pecan/hickory mix <sup>i</sup> , yellow pine <sup>j</sup> , pine mix <sup>l</sup> , common privet <sup>b</sup> , privet/olive mix <sup>b</sup> , queen palm, sweet gum, American sycamore <sup>k</sup> , sycamore mix <sup>k</sup> , western sycamore <sup>k</sup> , black walnut <sup>l</sup> , black willow <sup>f</sup>
<b>Grasses</b>
Bahia, Bermuda, blue <sup>l</sup> , brome <sup>l</sup> , Johnson, orchard <sup>l</sup> , quack <sup>l</sup> , red top <sup>l</sup> , red/sweet vernal mix <sup>l</sup> , fescue <sup>l</sup> , rye <sup>l</sup> , rye/fescue mix <sup>l</sup> , salt <sup>l</sup> , timothy <sup>l</sup>
<b>Weeds</b>
<i>Baccharis</i> sp., carelessweed, clover, cocklebur, daisy, dandelion, yellow dock <sup>m</sup> , dock mix/sheep sorrel mix <sup>m</sup> , dog fennel, English plantain, goldenrod, greasewood, <i>Kochi</i> sp., lambsquarter, marsh elder, mugwort <sup>n</sup> , mustard, nettle, rough pigweed <sup>o</sup> , pigweed mix <sup>o</sup> , short ragweed <sup>n</sup> , tall/giant ragweed <sup>n</sup> , false ragweed <sup>n</sup> , western ragweed <sup>n</sup> , ragweed mix <sup>n</sup> , Russian thistle, sagebrush mix, shadescale <sup>p</sup> , spearscale <sup>p</sup>
<b>Moulds</b>
<i>Alternaria tenuis</i> , <i>Aspergillus</i> mix, <i>Candida</i> sp., <i>Cephalosporium</i> sp., <i>Cladosporium</i> sp., <i>Curvularia/Dreschleria</i> spp., <i>Fusarium</i> sp., grass smut mix, <i>Helminthosporium</i> sp., <i>Mucor</i> mix, <i>Penicillium</i> mix, <i>Pullularia pullulans</i> , <i>Rhizopus nigricans</i> , <i>Stemphylium</i> sp.
<b>Mites and insects</b>
<i>Ascarus siro</i> <sup>q</sup> , <i>Bloma/Lepidoglyphus</i> mix <sup>q</sup> , black ant, cockroach, <i>Dermatophagoides pteronyssinus</i> <sup>q</sup> , <i>Dermatophagoides farinae</i> <sup>q</sup> , dust mite mix <sup>q</sup> , flea, fire ant, <i>Tyrophagus putrescentiae</i> <sup>q</sup>
<b>Epidermals</b>
Cat dander, human dander, sheep wool
*ACTT <sup>®</sup> (Biomedical Services), VARL Liquid Gold (Veterinary Allergy Reference Laboratory), ALLERCEPT <sup>®</sup> (Heska), Geer <sup>®</sup> Aller-g-complete (IDEXX)

\*Antigens that are grouped by cross-reactivity for the agreement analysis are denoted with a shared superscript letter.

For each dog, six pairwise laboratory comparisons (A:B, A:C, A:D, B:C, B:D and C:D) were made between the positive/negative test results in both the ungrouped antigen and grouped antigen analyses. The treatment recommendations from the laboratories were analysed in the same manner, comparing the agreement of recommended and nonrecommended allergens. Cross-tabulation agreement tables were produced for each pairwise comparison of laboratories. The agreement of the two laboratories in each table was expressed as a percentage agreement and chance-corrected agreement (Cohen's kappa,  $\kappa$ ).<sup>24</sup> While this report does present both percentage agreement and chance-corrected kappa agreement, the kappa agreement statistic is most relevant for assessing interlaboratory consistency in this study. Light's kappa (the average of all pairwise kappas, also denoted by  $\kappa$  in this text) was calculated for the overall pairwise agreement between all four laboratories for each dog.<sup>25</sup> For kappa calculations, only antigens or antigen groups common to each laboratory in the comparison could be included in the analyses. Two subsets of ungrouped antigens (plants and fungi in one subset and mites in the other) were also analysed separately for diagnostic agreement.

All statistical analyses were carried out in the statistical software R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria) version 2.15.2. Non-negative  $\kappa$  values were interpreted as follows, according to the categories defined by Landis and Koch: 0–0.20 as slight; 0.21–0.40 as fair; 0.41–0.60 as moderate; 0.61–0.80 as substantial; and 0.81–1 as almost perfect agreement.<sup>26</sup>

## Results

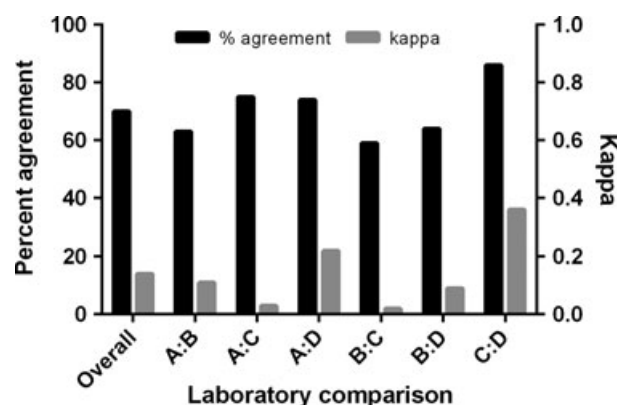
### Study population

Ten dogs were enrolled in the study between June 2012 and March 2013. Three Labrador retriever dogs and one each of the following breeds were represented: Boston terrier, English bulldog, French bulldog, German shepherd, German shorthaired pointer, Lhasa apso and mixed breed. One dog (dog 2) had received systemic glucocorticoid (prednisolone) treatment within 4 weeks of enrolment; the remainder had not. Results from Lab A allowed blinded determination of positive and negative allergens for all 10 dogs; however, Lab A declined to provide a

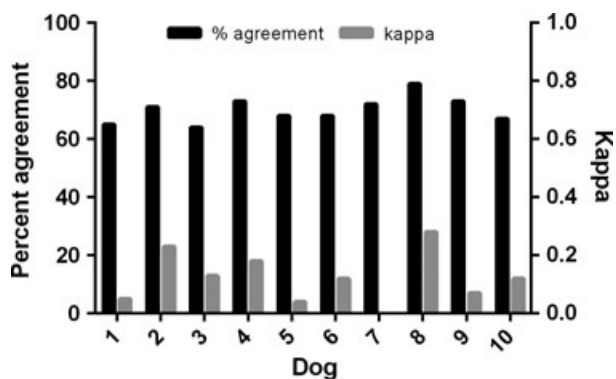
prescription recommendation for dog 4 after becoming aware of the study from an inadvertent communication from one of the investigators. For this reason, the data from dog 4 are not included in the ASIT recommendation agreement analysis. Serum samples from dogs 5–10 were subsequently collected and submitted to Labs A–D by a different investigator, whose participation remained concealed.

### Interassay diagnostic agreement

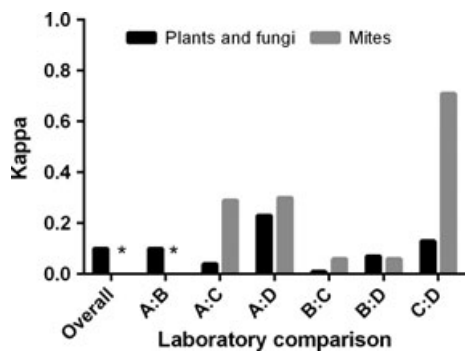
The overall diagnostic agreement across all dogs and all laboratories was 70% for ungrouped antigens (Figure 1), slightly higher than expected by chance (66%). When corrected for agreement by chance alone with the kappa statistic, the overall diagnostic agreement was



**Figure 1.** Overall and by laboratory pair, ungrouped allergen diagnostic percentage agreement and chance-corrected agreement (kappa statistic) of elevated allergen-specific IgE in 10 dogs with atopic dermatitis. Light's kappa is presented for overall agreement. Cohen's kappa is presented for laboratory comparisons. Laboratories are as follows: Lab A, Biomedical Services; Lab B, Veterinary Allergy Reference Laboratory; Lab C, Heska; and Lab D, IDEXX/Greer.



**Figure 2.** By dog, ungrouped allergen diagnostic percentage agreement and chance-corrected agreement (Light's kappa statistic) of elevated allergen-specific IgE in 10 dogs with atopic dermatitis.



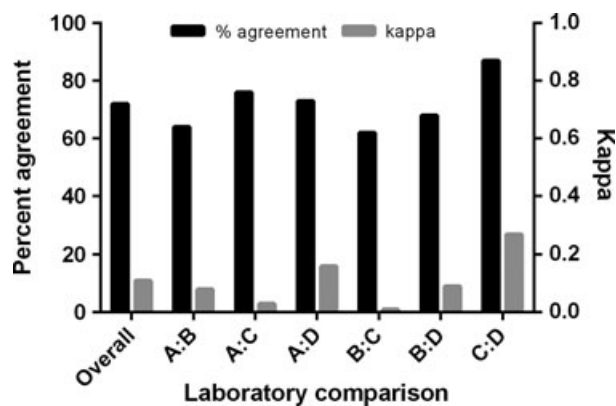
**Figure 3.** Overall and by laboratory pair diagnostic agreement for positive versus negative allergen-specific IgE detection of ungrouped plant/fungal and mite antigens in dogs with atopic dermatitis. Light's kappa is presented for overall agreement. Cohen's kappa is presented for laboratory comparisons. Laboratories are as in Figure 1. \*Could not be calculated because no identical mite antigens were evaluated by Lab A and Lab B.

slight ( $\kappa = 0.14$ ; Figure 1). The diagnostic agreement by dog (Figure 2) and by laboratory pair (Figure 1) can also generally be characterized as slight for the majority of the agreement comparisons. Similar percentage agreement (58–82%) and kappa values (0.01–0.28) were obtained when the overall, by-laboratory pair and by-dog analyses were repeated with antigens grouped by taxonomy, cross-reactivity and co-sensitization (Figures S1 and S2 in Supplementary material).

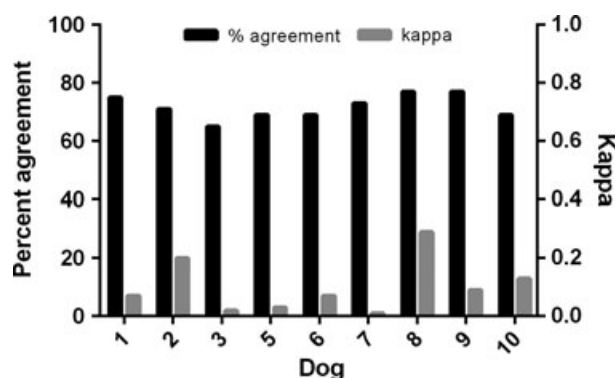
The best chance-corrected agreement was found between Labs C and D ( $\kappa = 0.36$ , fair). Nine of the 12 positive agreements between Labs C and D across the 10 dogs were for mites. Subset analysis of Labs C and D found 86% agreement ( $\kappa = 0.13$ , slight) for ungrouped plants and fungi, and 87% agreement ( $\kappa = 0.71$ , substantial) for ungrouped mites (Figure 3). The  $\kappa$  values can be characterized as slight or fair for all other between-laboratory comparisons of diagnostics on ungrouped antigens limited to either plants and fungi or mites (Figure 3).

#### Interlaboratory ASIT recommendation agreement

The overall ASIT recommendation percentage agreement across nine dogs and all laboratories was 72% for ungrouped allergens (Figure 4). This is only slightly higher percentage agreement than would be expected



**Figure 4.** Overall and by laboratory pair, ungrouped allergen treatment recommendation percentage agreement and chance-corrected agreement (kappa statistic) in nine dogs with atopic dermatitis. Light's kappa is presented for overall agreement. Cohen's kappa is presented for laboratory comparisons. Laboratories are as in Figure 1.



**Figure 5.** By dog, ungrouped allergen treatment recommendation percentage agreement and chance-corrected agreement (Light's kappa statistic) in nine dogs with atopic dermatitis.

by chance (69%). The overall ASIT recommendation agreement as analysed with the kappa statistic was only slightly better than expected by chance ( $\kappa = 0.11$ ; Figure 4). Agreement percentage by dog (Figure 5) and by laboratory pair (Figure 4) ranged from 62 to 87% for ungrouped antigens. The chance-corrected agreement of ASIT recommendations by dog and by laboratory pair was generally slight. Similar ranges of percentage agreement (59–83%) and chance-corrected agreement ( $\kappa = 0.01$ –0.27) were found when the ASIT recommendations were analysed by antigen groups (Figures S3 and S4 in Supplementary material). The overall and by laboratory pair  $\kappa$  values were very similar ( $\pm 0.02$ ) when the analysis was repeated for all 10 dogs with the 10 positive reactions for dog 4 in Lab A's assay defined as 'recommended' (data not shown). The highest level of chance-corrected ASIT recommendation agreement was found between Labs C and D (Figure 4;  $\kappa = 0.27$ , fair agreement).

#### Descriptive data

Among the six pairs of laboratory comparisons, the number of shared antigens available for interassay comparison of a pair ranged from 233 to 316 for ungrouped antigens, and from 239 to 335 for grouped antigens

**Table 2.** The number of antigens resulting in positive allergen-specific IgE findings by four laboratories evaluating replicate serum samples from dogs with atopic dermatitis

Dog no.	Lab A	Lab B	Lab C	Lab D
No. of antigens tested	61–63	40	48	48
1	25	17	0	1
2	14	14	3	15
3	21	13	4	19
4	10	14	4	10
5	11	14	0	10
6	9	15	3	13
7	9	14	0	1
8	15	6	4	4
9	12	15	0	3
10	17	15	2	4
Mean (% of tested)*	14.3 (23%)	13.7 (34%)	2.0 (4.2%)	8.1 (17%)

\*The mean was calculated by the averaging the positive allergen-specific IgE findings across dogs. The percentage of tested was calculated as the ratio of the number of the positive allergen-specific IgE findings pooled across all 10 dogs divided by the number of all tests for all 10 dogs  $\times$  100%. Laboratories are as follows: Lab A, Biomedical Services; Lab B, Veterinary Allergy Reference Laboratory; Lab C, Heska; and Lab D, IDEXX/Greer.

(ranging across all six pairs of laboratories and pooling data across the 10 dogs.)

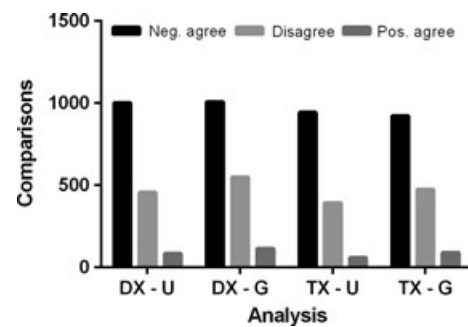
The prevalence of positive reactions across all dogs for each laboratory ranged from 4.2 to 34% of antigens tested (Table 2). The number of positive reactions per laboratory for each dog varied widely. Four of the 10 dogs (dogs 1, 5, 7 and 9) did not have any positive results reported by Lab C. None of these had received glucocorticoid therapy for at least 4 weeks before serum sampling. The same four dogs had a mean of 11 positive results per dog reported by the other three laboratories.

Forty-four allergens were assigned an ordinal score of '1' by Lab B for the 10 dogs (median, 4.5 per dog). Of these, three were recommended for immunotherapy by Lab B despite falling below the positive/negative cut-off value we defined as positive, in accordance with methodology previously reported by this laboratory.<sup>13</sup> All three of these antigen recommendations were made for a single dog (dog 8), which had the lowest number of positive reactions for Lab B amongst the 10 dogs (Table 2).

The number of diagnostic and ASIT recommendation disagreements was very large compared with the number of positive agreements (Figure 6). For the nine dogs for which all four laboratories provided treatment recommendations, 261 antigens were recommended for ASIT by the laboratories collectively. Of these antigens, 85.1% were recommended by one laboratory or another (without recommendation by a comparison laboratory), 11.5% by two laboratories, 3.1% by three laboratories and 0.4% by all four laboratories (the sum does not equal 100.0% due to rounding).

## Discussion

Our results show that the agreement of four allergen-specific IgE assays, while considerable as measured by percentage agreement, is only slightly better than could



**Figure 6.** Total number of pairwise agreements and disagreements between four serum allergen testing laboratories for assays on 10 dogs. Abbreviations: DX, positive/negative diagnostic agreement; G, grouped antigens with phylogenetically related, cross-reactive and cross-sensitizing antigens considered equivalent; Neg, negative; Pos, positive; TX, include/exclude treatment recommendation agreement; U, ungrouped antigens evaluated as tested.

be expected by chance. This counterintuitive result is possible when a high percentage of results are negative, as was the case in our study. The number of negative pairwise agreements and the number of disagreements greatly exceeded the number of positive agreements (Figure 6).

Previous studies have measured the interlaboratory and interassay agreement of the allergen-specific IgE assays used by Labs C and D.<sup>14–16</sup> The concordance (percentage agreement) of positive/negative results exceeded 90%, leading the authors to conclude that the assay results were comparable, but the statistical methodology did not account for agreement that might have occurred by chance alone. In addition to the difference in statistical methodology, another possible explanation as to why our findings differed from those of Lee *et al.*<sup>15</sup> is a difference in the nature of the serum samples used in the respective studies.<sup>15</sup> Discordant allergen-specific IgE results are more likely to occur at levels close to those defined as the cut-off values for each assay.<sup>15</sup> In our study, aliquots of 10 individual blood samples from clinical patients diagnosed with atopic dermatitis were submitted to each laboratory. In an interassay comparison,<sup>15</sup> 18 serum pools were prepared from individual samples with known macELISA allergen reactivity in order to generate a wide spectrum of reactivity, from negative to highly positive.<sup>15</sup> This may have reduced the prevalence of borderline allergen-specific IgE levels in comparison to those found in our individual serum samples.

Laboratory comparisons for indications with very high or very low prevalence can result in a high percentage agreement rate, merely by chance, while the chance-corrected agreement may be low. As a hypothetical example with features similar to those encountered in this study, suppose that Lab X and Lab Y analyse 100 allergens, agree negative on 80 allergens, agree positive on two allergens, have nine allergens positive for Lab X and negative for Lab Y, and nine allergens positive for Lab Y and negative for Lab X. For these data, the prevalence is a low 11%, the agreement rate is a high 82%, while the chance-corrected kappa agreement is a very low 0.08. Among the 20 allergens in this example where either or both laboratories yielded a positive result, there were only

two allergens where the two laboratories agreed positive, which is clearly poor agreement. If Labs X and Y randomly and independently assigned allergens to positive or negative status in a manner to retain the 11% positive prevalence for each laboratory, the expected agreement would be 80%, which is not very different from the observed 82% agreement rate. The percentage agreement rate includes potentially random agreement, whereas kappa excludes it. It seems relevant to measure agreement as a correspondence between laboratories that goes beyond chance agreement, and that is the role of kappa. Thus, we report the chance-corrected agreement with the kappa statistic along with percentage agreement for four serum IgE assays and ensuing treatment recommendations.

The highest level of chance-corrected agreement occurred between Labs C and D. Labs C and D displayed substantial chance-corrected agreement (kappa) on diagnostic results for mite antigens, but only slight chance-corrected agreement for plant and fungal antigens. The reproducibility of SAT has previously been shown to differ amongst allergen groups.<sup>5,16</sup> The interlaboratory coefficient of correlation was notably lower for fungal than for mite, grass, tree or weed allergens in a study comparing six European laboratories using the macELISA.<sup>16</sup>

In order for two or more assays of allergen-specific IgE to display a high level of interassay agreement, each must have a high degree of intra-assay reproducibility. Whether the poor interassay agreement observed for the 10 dogs in this study was attributable to intra-assay or interassay variability was not determined. Laboratories offering SAT face significant technical challenges that may contribute to intra-assay variability. Potential pitfalls include nonspecific anti-IgE antibody binding, allergenic extract variability, nonspecific signal molecule activation and establishing clinically relevant cut-off values.<sup>6</sup> The assays evaluated in the present study use a variety of methodologies, employing different types of IgE detection methods and enzyme conjugates, each presenting unique technical challenges. As natural products, allergens may differ by source and by batch. This remains the greatest challenge to harmonizing different allergen-specific IgE assays in human medicine.<sup>27</sup> It was beyond the scope of the present study to investigate the numerous potential causes of the observed laboratory disagreement. Our findings emphasize the need for an independent, interlaboratory reliability monitoring programme for canine allergen-specific IgE assays, as has been previously proposed.<sup>14,15</sup>

Allergen-specific serum IgE assays are considered to be a valid means of identifying allergens for possible inclusion in ASIT extracts, based on the observation that ASIT produces a similar clinical benefit for canine AD whether based on SAT or IDT.<sup>3,7,28–30</sup> Although the laboratories evaluated in this report are located in the USA, Labs A and B process samples from outside the USA, and the assays used by Labs C and D are used in European laboratories.<sup>14–16</sup> Despite the widespread use of the assays and presumably the ASIT recommendations provided by companies marketing them, the reproducibility of ASIT prescription recommendations has not been evaluated. We found that the overall agreement of the laboratories' ASIT

prescription recommendations is only slightly better than could be expected by chance. Our findings emphasize the importance of considering clinical factors beyond elevated allergen-specific IgE levels (e.g. likely allergen exposure) in formulating ASIT.<sup>2</sup>

Lab C did not make ASIT recommendations for four of 10 dogs due to low levels of reactivity on the ALLERCEPT<sup>®</sup> assay (Table 2). These same dogs often had 10 or more positive reactions according to the other three assays. Overall, the VARL Liquid Gold assay resulted in the highest prevalence (34%) and the ALLERCEPT<sup>®</sup> assay the lowest prevalence (4.2%) of positive reactions across the 10 dogs. Whether this stark difference is due to allergen selection, false-positive reactions or false-negative results is uncertain, but it would clearly influence ASIT recommendations and, perhaps, whether the dogs would be diagnosed with 'atopic-like dermatitis', without a demonstrable IgE response, rather than 'atopic dermatitis'.<sup>31</sup>

The clinical implications of the poor prescription recommendation agreement on ASIT efficacy are uncertain, but our results suggest that the choice of serum IgE assay is not a trivial matter. It is possible that one laboratory's recommended prescription is consistently more efficacious than another's, but this is not necessarily the case. We did not evaluate the efficacy of the recommended ASIT prescriptions, nor intralaboratory reliability, which could influence the consistency of the recommendations by a single laboratory. Furthermore, while it is implicitly assumed in the term 'allergen-specific' that an optimal allergen formulation for each atopic dog exists, this may not be true. Evidence to the contrary is as follows: (i) some beneficial effects of immunotherapy are nonspecific; (ii) ASIT based on varying testing methodologies results in similar efficacy; and (iii) standardized immunotherapy mixtures and those customized based on allergen test results may be of similar efficacy.<sup>3,7,28–30,32–34</sup> Robust studies are needed to compare the efficacy of ASIT based on different allergenic extract formulation methods.

There were substantial differences between the four laboratories in the allergens included in regional assays from the same geographical region, which is likely to have influenced the number of unique allergens recommended for immunotherapy. Our agreement analysis, however, was limited to the individual allergens (ungrouped analysis) or allergen groups (grouped analysis) common to both assays in each pairwise comparison. Cross-reactivity or co-sensitivity within allergen groups has been demonstrated in atopic dogs.<sup>22,35</sup> In our study, the pairwise agreement results of phylogenetically related, cross-reactive or co-sensitizing grouped antigens were generally similar to those found for the ungrouped antigens; therefore, the poor agreement between laboratories did not appear to stem from the standard panels of the four laboratories incorporating different, but similar antigens. However, the allergen groupings we used in our analysis were based largely on data from humans, which may differ from their behaviour in dogs.

We evaluated the agreement of the results in binary terms (positive or negative reactivity), which may differ from the agreement of continuous EAU scales, when

available. The cut-off levels established by each laboratory were used in our study to reflect the manner in which practising veterinarians most often differentiate positive from negative results.<sup>14</sup> Using different cut-off levels could have led to different levels of agreement.<sup>15</sup>

A limitation of our study is that we relied on the treatment recommendations obtained from the laboratories without question, while in practice, the prescribing veterinarian is free to ignore or modify these recommendations. It is possible that veterinarians in practice do not rely heavily on the laboratories' treatment recommendations, but this is unlikely. Prescription recommendations are either provided routinely or upon request by all four of the laboratories evaluated.

The small number of dogs included in this study is another limitation. With a properly designed study including a larger number of dogs, one should be able to establish a more statistically rigorous inference for the general population of dogs and laboratories, including 95% confidence intervals for the  $\kappa$  and agreement estimates. Although our study was limited to 10 dogs, the chance-corrected agreement was undesirably low for each of them. Agreement by dog was generally characterized only as slight for both grouped antigens and ungrouped antigens. This was true of both diagnostic and ASIT treatment recommendation agreement. The low level of between-laboratory agreement on 261 allergen treatment recommendations in nine dogs (compared with what would be expected merely by chance) is also striking. If the findings from the 10 dogs in our study are representative of a wider population of atopic dogs, it draws into question the comparability of these four allergen-specific IgE assays.

In summary, the chance-corrected agreement of four commercially available allergen-specific IgE assays was evaluated for 10 dogs with atopic dermatitis. The results show that the overall interassay agreement was only slightly better than expected by chance. No two laboratories displayed even moderate chance-corrected agreement ( $\kappa > 0.40$ ) with each other. The ASIT recommendations provided by the laboratories for nine dogs also displayed slight overall agreement. Eighty-five per cent of the 261 ASIT antigen recommendations for these nine dogs were unique to one laboratory or another. Further studies are required to determine the degree to which the choice of allergen testing laboratory impacts on the efficacy of ASIT.

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## Résumé

**Contexte** – Les tests d'IgE spécifiques d'allergènes chez le chien aux USA ne sont pas soumis à un programme de surveillance fiable par un laboratoire indépendant.

**Hypothèses/Objectifs** – Le but de cette étude était d'évaluer l'harmonie des résultats diagnostics et des recommandations de traitement de quatre tests sériques d'IgE disponibles dans le commerce aux USA.

**Méthodes** – Les échantillons de sérum de 10 chiens atopiques ont été soumis à chacun des quatre laboratoires pour tests d'IgE spécifiques d'allergènes (ACTT<sup>®</sup>, VARL Liquid Gold, ALLERCEPT<sup>®</sup> et Greer<sup>®</sup> Aller-g-complete<sup>®</sup>). L'harmonie inter-laboratoire des panels régionaux, standards et des recommandations de traitements qui en découlent ont été analysés par statistique kappa ( $\kappa$ ) pour évaluer comme accord ce qui pourrait arriver simplement par hasard. Six comparaisons de paires de laboratoires et d'harmonie globale parmi les laboratoires ont été analysées pour les allergènes non groupés (comme testés) et aussi les allergènes groupés conformément à la réactivité croisée rapportée et à la taxonomie.

**Résultats** – L'accord de chance corrigée totale des résultats des tests positifs/négatifs pour les allergènes non-groupés et groupés était minime (respectivement  $\kappa = 0.14$  et  $0.13$ ). L'analyse des sous-groupes des paires de laboratoires avec un haut niveau d'accord de diagnostic ( $\kappa = 0.36$ ) a montré une harmonie modérée ( $\kappa = 0.13$ ) pour les plantes et les champignons non-groupés mais une harmonie substantielle ( $\kappa = 0.71$ ) pour les acariens non-groupés. L'accord global des recommandations de traitement était modérée ( $\kappa = 0.11$ ). Dans l'ensemble, 85.1% des recommandations de traitement d'allergènes non-groupés étaient unique d'un laboratoire à l'autre.

**Conclusions et importance clinique** – Notre étude indique que le choix des tests d'IgE peut avoir une influence majeure sur les résultats positifs ou négatifs et les recommandations de traitements qui en découlent.

## Resumen

**Introducción** – las pruebas alérgicas específicas de IgE canina en los EEUU no están sujetas a un programa independiente de evaluación laboratorial.

**Hipótesis/Objetivos** – el propósito de este estudio fue evaluar el grado de concordancia en los resultados diagnósticos y las recomendaciones de tratamiento de cuatro pruebas de detección de IgE canina del suero disponibles en EEUU.

**Métodos** – muestras replicadas de suero de 10 perros con atopia se remitieron a cada uno de los cuatro laboratorios para pruebas alérgicas específicas de detección de IgE (ACTT<sup>®</sup>, VARL Liquid Gold, ALLERCEPT<sup>®</sup>

## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Figure S1.** Overall and by laboratory pair, grouped allergen diagnostic percentage agreement and chance-corrected agreement (kappa statistic) of elevated allergen-specific IgE in 10 dogs with atopic dermatitis. Light's kappa is presented for overall. Cohen's kappa is presented for laboratory comparisons.

**Figure S2.** By dog, grouped allergen diagnostic percentage agreement and chance-corrected agreement (Light's kappa statistic) of elevated allergen-specific IgE in 10 dogs with atopic dermatitis.

**Figure S3.** Overall and by laboratory pair, grouped allergen treatment recommendation percentage agreement and chance-corrected agreement (kappa statistic) in nine dogs with atopic dermatitis. Light's kappa is presented for overall. Cohen's kappa is presented for laboratory comparisons.

**Figure S4.** By dog, grouped allergen treatment recommendation percentage agreement and chance-corrected agreement (Light's kappa statistic) in nine dogs with atopic dermatitis.

**Table S1.** Antigens assayed by each of four laboratories in regional panels for 10 dogs from southern Texas, USA.



y Greer® Aller-g-complete®). Se analizaron las concordancias entre laboratorios de los paneles estándar y regionales así como las recomendaciones de tratamiento con la prueba estadística kappa ( $\kappa$ ) para valorar el nivel de concordancia que puede ocurrir simplemente por casualidad. Seis comparaciones pareadas entre laboratorios y la concordancia global entre laboratorios se analizaron para alérgenos no agrupados (según la prueba) y también para alérgenos agrupados de acuerdo con la reactividad cruzada publicada y la taxonomía.

**Resultados** – la concordancia global corregida por azar de los resultados positivos/negativos de las pruebas para alérgenos no agrupados y agrupados fue pequeña ( $\kappa = 0,14$  y  $0,13$ , respectivamente). Un análisis en menor escala del par de laboratorios con mayor concordancia en el diagnóstico ( $\kappa = 0,36$ ) encontró poca concordancia ( $\kappa = 0,13$ ) para plantas no agrupadas y hongos, pero una concordancia importante ( $\kappa = 0,71$ ) para ácaros no agrupados. La concordancia global de las recomendaciones de tratamiento fue pequeña ( $\kappa = 0,11$ ). En total, 85,1% de las recomendaciones de tratamiento de alérgenos no agrupados fueron únicas para un laboratorio concreto.

**Conclusiones e importancia clínica** – nuestro estudio indica que la elección de la prueba de IgE tiene un impacto importante en los resultados positivos/negativos y en las recomendaciones de tratamiento.

### Zusammenfassung

**Hintergrund** – Canine allergenspezifische IgE Assays unterliegen in den USA keinem unabhängigen Monitoring in Bezug auf ihre Verlässlichkeit.

**Hypothese/Ziele** – Das Ziel dieser Studie war eine Evaluierung der Übereinstimmung der diagnostischen Ergebnisse und der Behandlungsempfehlungen vier kommerziell verfügbarer Serum IgE Assays in den USA.

**Methoden** – Replizierte Serumproben von 10 atopischen Hunden wurden jeweils an vier Laboratorien zur Durchführung eines allergen-spezifischen IgE Assays (ACTT®, VARL Liquid Gold, ALLERCEPT® und Greer® Aller-g-complete®) gesendet. Die Interlabor-Übereinstimmungen bzgl. Standard, regionaler Paneele und daraus resultierender Behandlungsempfehlungen wurden mittels kappa Statistik ( $\kappa$ ) analysiert, um eine eventuell zufällige Übereinstimmung zu erkennen. Es wurden sechs Vergleiche von je zwei Laboratorien und allgemeine Übereinstimmungen unter den Laboratorien auf nicht gruppierte Allergene (wie sie getestet wurden) und auch gruppierte Allergene im Einklang mit ihrer beschriebenen Kreuzreaktivität und Taxonomie analysiert.

**Ergebnisse** – Die allgemeine auf Zufall-korrigierte Übereinstimmung der positiven/negativen Testergebnisse für ungruppierte und gruppierte Allergene war gering ( $\kappa = 0,14$  bzw.  $0,13$ ). Eine Untergruppenanalyse der beiden Laboratorien mit den höchsten diagnostischen Übereinstimmungen ( $\kappa = 0,36$ ) ergab eine geringe Übereinstimmung ( $\kappa = 0,13$ ) für ungruppierte Pflanzen und Pilze, aber eine deutliche Übereinstimmung ( $\kappa = 0,71$ ) für ungruppierte Milben. Die allgemeine Übereinstimmung der Behandlungsempfehlungen waren gering ( $\kappa = 0,11$ ). Zusammengefasst waren die Behandlungsempfehlungen von 85,1% aller ungruppierten Allergene bei den jeweiligen Laboratorien unterschiedlich.

**Schlussfolgerungen und klinische Bedeutung** – Unsere Studie zeigte, dass die Wahl eines IgE Assays einen großen Einfluss auf positiv/negative Testergebnisse und die daraus resultierenden Behandlungsempfehlungen haben kann.

### 摘要

**背景** – 美国的独立实验室进行的犬特异性 IgE 过敏原试验，并不是可靠的检测项目。

**假设/目的** – 此研究的目标是评估美国四家商业实验室的 IgE 试验诊断结果与治疗的一致性。

**方法** – 采集 10 只异位性皮炎患犬血液的重复样本，分别送到 4 家实验室 (ACTT®, VARL Liquid Gold, ALLERCEPT® and Greer® Aller-g-complete®)。进行犬特异性 IgE 过敏原试验。多个实验室的标准一致性、地域过敏原组合及随后的治疗建议，都用卡巴统计量 ( $K$ ) 进行分析，以说明一致性的存在是偶然因素。实验室配对的六对比较，及所有结果的一致性进行分析，包括未予分组的过敏原（已测试）以及那些根据报道有交叉反应和分类学一致的分组过敏原。

**结果** – 阳/阴性试验结果总的偶然性矫正，不论抗原分组与否都轻微一致 ( $K=0.14; 0.13$ )。两实验室对的诊断一致性分析 ( $K=0.36$ )，一致性最高程度在未分组植物和真菌结果轻微一致 ( $K=0.13$ )，但未分组的螨虫结果大体一致 ( $K=0.71$ )。总体治疗轻微一致 ( $K=0.11$ )。总之，各实验室间 85.1% 的未分组过敏原治疗完全不同。

**结论及临床意义** – 本研究表明，IgE 试验的实验室选择可能会对阳/阴测试结果及后续治疗产生重要影响。

## 要約

**背景** – アメリカ合衆国においてイヌアレルギー特異的IgE検査は独立した実験的な信頼性監視プログラムを受けていない。

**仮説/目的** – この研究の目的はアメリカ合衆国において商業的に利用可能な4つの血清IgE検査の診断的結果、および推奨される治療の合意を評価することである。

**方法** – 10頭のアトピー犬からの複製した血清材料をアレルギー特異的IgE検査のため4つの研究所 (ACTT®, VARL Liquid Gold, ALLERCEPT® および Greer® Aller-g-complete®) にそれぞれ送付した。研究所間において偶然によって生じる可能性のある合意を説明する為に基準、アレルギー検査項目ならびにその後の治療推奨を  $\kappa$  統計量で解析を行った。研究所を対にした6つの比較および研究所間の全体的な合意を非グループ化した (テストを実施した) アレルギー、および報告された交差反応性や分類に基づいてグループ化したアレルギーで解析を行った。

**結果** – 非グループ化した、およびグループ化したアレルギーに対する陽性/陰性の検査結果における全体的な偶然を補正した合意はわずかであった (それぞれ  $\kappa = 0.14$  および  $0.13$ )。最も高いレベルの診断的合意 ( $\kappa = 0.36$ ) について、研究所の組み合わせにおけるサブセット解析では非グループ化した植物および菌類においてわずかな合意 ( $\kappa = 0.13$ ) がみられたが、非グループ化したダニにおいては十分な合意がみられた ( $\kappa = 0.71$ )。結局は、85.1%の非グループ化したアレルギー治療の推奨は1つの研究所またはその他に対して特有であった。

**結論および臨床的な重要性** – 筆者らの研究はIgE検査の選択は陽性/陰性の結果ならびに結果として生じる治療の推奨に大きな影響を示す可能性を示唆していた。