



## ELISA FOR ALLERGEN-SPECIFIC IgE DETERMINATION IN DOG. CORRELATION WITH *IN VIVO* DIAGNOSTICS

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### INTRODUCTION

Diagnosis of IgE-mediated hypersensitivities in dogs is based on the clinical history of the animal, physical examination and the results from allergy tests to a battery of allergens that correlate with clinical history. Intradermal skin test (IDST) has long been considered the "gold standard" among these tests. However, there are important drawbacks for the practical execution of this technique as compared to "in vitro" tests. Recently, a new enzyme-linked immunosorbent assay (PET-ELISA) to measure allergen-specific IgE in dog serum has been developed by ALERGOVET S.L.

### OBJECTIVES

To study the correlation of the levels of specific IgE against grass pollen allergens, as determined by PET-ELISA, with the results obtained *in vivo* IDST in a group of grass-allergic dogs.

### MATERIALS AND METHODS

#### • Patients:

Forty-one dogs with clinical histories suggestive of atopy and positive IDST to grasses were selected and classified in three groups according to the intensity of the cutaneous reactions, the number of positive reactions to a standard battery of inhalant allergens (Stallergenes S.A. and C.B.F.Leti S.A.) and the clinical history.

#### • ELISA for specific IgE to grass pollens:

Specific IgE to grass pollens in serum of patients was determined using the PET-ELISA for Grass mix I (*Lolium perenne*, *Phleum pratense*, *Dactylis glomerata*, *Festuca elatior* and *Poa pratensis*). Briefly, plastic microtiter wells are coated with the pollen extracts and sequentially incubated with the serum samples, a biotinylated polyclonal rabbit anti-IgE, peroxidase-conjugated streptavidin, and a chromogen substrate for peroxidase (*orto*-phenylenediamine). The intensity of the colour produced in the enzymatic reaction is proportional to the amount of specific IgE present in the serum sample. The level of specific IgE is established by comparison with the colour intensity produced by previously calibrated controls, and expressed as classes: negative (0), borderline (1), weak positive (2), positive (3) and strong positive (4).

**Table 1. Description of the three groups of patients and a control group**

GROUP	Nº	Grasses IDST	Symtoms
A	17	3+, 4+	Atopic pattern of pruritus. Intense seasonal peaks.
B	16	2+, 3+	Moderate to weak pruritus. Seasonal peaks.
C	8	1+, 2+	Uncertain atopy. Weak pruritus. No seasonal peaks.
Control	16	0	Negative history of atopy.

## RESULTS AND DISCUSSION

The results obtained in the PET-ELISA for Grass Mix I, expressed as IgE classes, are indicated in Table 2 for each group of patients.

Only 3 out of the 24 dogs included in groups C and control gave a weak positive result (Class 2) in the specific IgE ELISA, and none of them were Class 3 or 4. In the groups made up of clearly atopic animals (A and B), eight patients, from a total of 33, produced results in the 0-1 classes in the ELISA; five of these patients were borderline.

Table 3 displays the predictive value parameters commonly used to evaluate a laboratory test: sensitivity, specificity, the predictive values of positive and negative results, and the diagnostic efficiency of the test.

From the results in these tables, it can be inferred that the ELISA for allergen specific IgE determination clearly differentiates allergic from non-allergic animals. Thus, the specificity of the assay is very high (81.3%), and so is the sensitivity (82.9%). Moreover, the intervals chosen to define the different classes of specific IgE showed a very good correspondence with the criteria used for the classification of patients in subgroups, which included clinical histories. Thus, dogs in group A were almost homogeneously distributed around Class 3, and those in group B were mostly clustered around Class 2.

Although a perfect correlation between specific IgE ELISA classes and IDST scores should not be expected, as these techniques do not measure exactly the same magnitude (the ELISA detects circulating IgE antibodies, whereas IDST detects IgE bound to mast cells), a very good concordance have been observed between both diagnostic methods. As mentioned above, the classification of animals in different groups had been established on the basis of the clinical history of atopy and IDST results. Nevertheless, it is known that all the diagnostic methods in allergy can identify hypersensitivities but these do not necessarily imply a clinical significance. Thus, one of the main features of the IDST is its extreme sensitivity, producing positive reactions even in sub-clinical hypersensitivity states. Taking this fact into account, we could consider negative the group C in our study, with a questionable diagnostic of atopy, and, in the same way, we could consider negative the results of class 1 (borderline) in ELISA. In this case, the specificity of the assay would be even greater (87.5%, see values in parenthesis in Table 3).

**Table 2. Results of specific IgE ELISA in the four groups of dogs.**

GROUP	Specific IgE				
	Class 0	Class 1	Class 2	Class 3	Class 4
A	0	2	4	8	3
B	3	3	7	1	2
C	4	3	1	0	0
Control	13	1	2	0	0

**(Results are expressed as specific IgE classes according to the score defined in Materials and Methods).**

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Sensitivity	Specificity	Predictive value of		Efficiency
		Positive test	Negative test	
82.9 (75.8)	81.3 (87.5)	91.9 (89.3)	65.0 (72.4)	82.5 (80.7)

## CONCLUSIONS

In this study, we have demonstrated that the PET-ELISA for allergen-specific IgE in dog has a high degree of sensitivity (83%) and specificity (81%) as compared with the IDST and, in addition, it shows a very good correspondence with the clinical histories of atopy of patients.