

Known IgE, Different Answers

Analytical Performance of Three Canine Serum Allergen Tests

Evaluated Using Artificial Sera with Predefined Allergen-Specific Canine IgE Monoclonal Antibodies

FREJA KROGH*, LENE BOYSEN*
*Vetgruppen Birkerød Dyrehospital, Birkerød, Denmark

Introduction

Serum allergen testing (SAT) is widely used to detect allergen-specific IgE in dogs with atopic dermatitis and to guide allergen immunotherapy (AIT). However, comparison of available assays is limited by the fact that naturally sensitized canine sera do not have a known IgE composition. When laboratories report different sensitization profiles, it makes it difficult to determine whether the variation reflects true sensitizations or assay-related differences. The aim of this study was to use artificial sera with predefined allergen-specific monoclonal dog IgE to directly assess the analytical performance of three commonly used SAT methods.

Methods

Artificial sera were prepared from fetal bovine serum and spiked with laboratory-generated allergen-specific monoclonal dog IgE targeting Phl p 5 (*Timothy grass*), Der f 2 (*D. farinae*) and Pla a 3 (*Platanus*). Eleven serum mixes were prepared, including one negative control consisting of Der f 2-specific dog IgG-B (IgG1) to test if the assays wrongly measure IgG. The samples were randomized and submitted blindly by an external investigator to three European laboratories using different methods for serum IgE detection: a molecular allergology multiplex macroarray (PAX; Nextmune AB, Sweden), a monoclonal antibody cocktail ELISA (MAC; IDEXX Laboratories, Hoofddorp, The Netherlands) and an Fcε-receptor-based ELISA (Allercept; LABOKLIN, Bad Kissingen, Germany). Results were interpreted according to manufacturer thresholds and compared with the predefined IgE composition using sensitivity, specificity, percentage agreement, Cohen’s kappa and coefficient of variation.

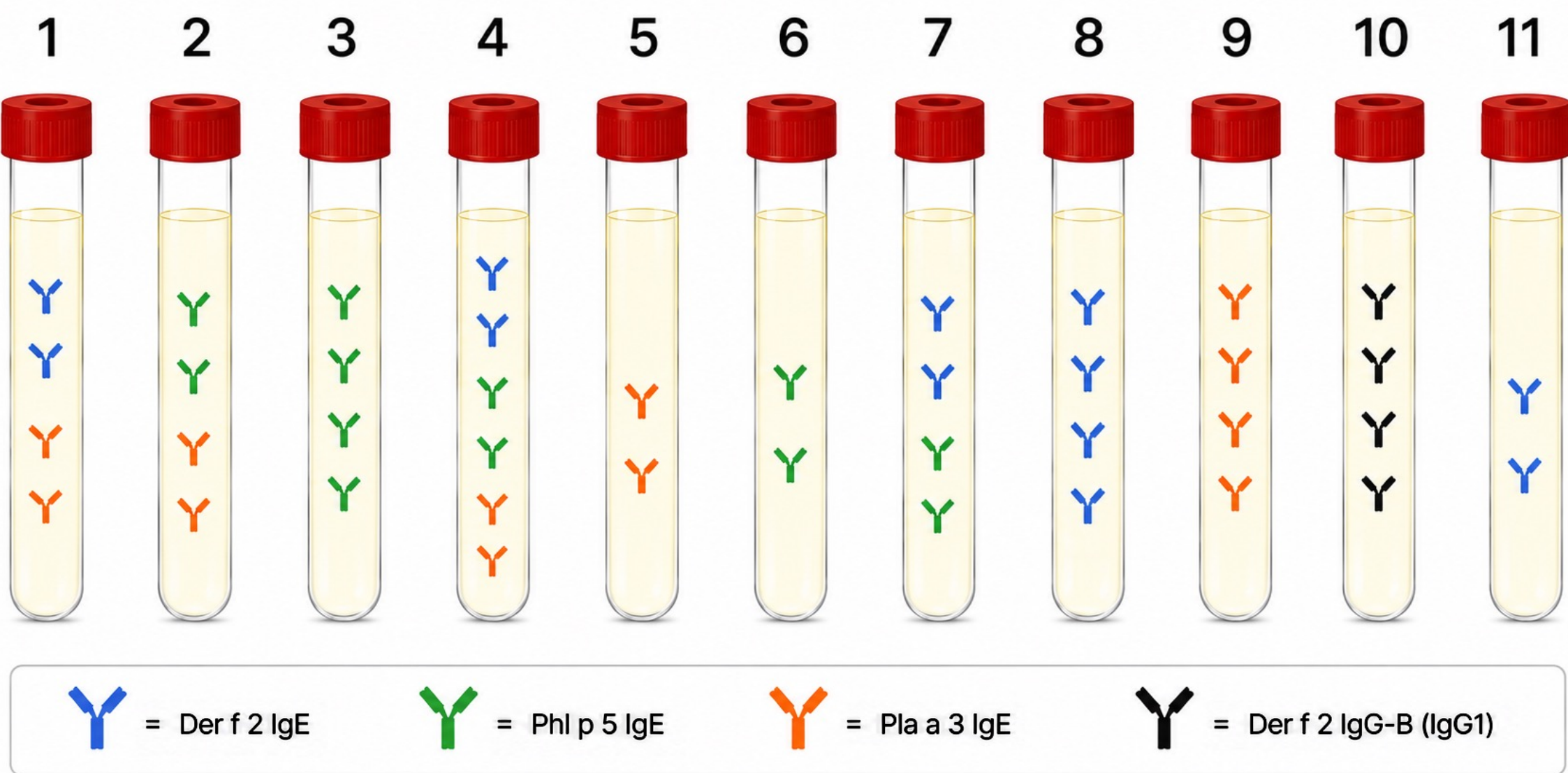


Figure 1. IgE depleted fetal bovine serum spiked with predefined combinations of Der f 2, Phl p 5, and Pla a 3 monoclonal dog IgE. Tube 10: Negative control consisting of serum spiked with Der f 2 dog IgG-B (IgG1).

Performance Measure	PAX	MAC	Allercept
% correctly classified samples	100%	27%	18%
Cohen’s kappa	1	0.353	0.113
Sensitivity	100%	33%	70%
Specificity	100%	100%	42%
CV% <i>Dermatophagoides farinae</i> (Der f 2)	1.8%	144.2%	200%
CV% <i>Timothy grass</i> (Phl p 5)	7.4%	28%	12.8%
CV% <i>Sycamore</i> (Pla a 3)	5%	N/A	N/A

Figure 2. Summary of qualitative and quantitative performance of three serological assays evaluated on 11 serum samples spiked with predefined combinations of allergen-specific IgE monoclonal antibodies. Performance measures include percentage of correctly classified samples, Cohen’s kappa (κ), sensitivity, specificity, and coefficient of variation (CV%) calculated from four independent sera containing identical low concentrations of IgE monoclonal antibodies. N/A indicates that the allergen was not included in the respective assay panel.

Results

The three assays differed markedly in their ability to reproduce the expected IgE profiles. The PAX correctly classified all 11 serum samples, resulting in 100% sensitivity, 100% specificity and complete agreement with the predefined composition (κ = 1.0). The MAC correctly classified 3 of 11 samples, with reduced sensitivity (33%) but high specificity (100%) and moderate agreement (κ = 0.353). The Allercept correctly classified 2 of 11 samples and showed both false-negative and false-positive reactions, resulting in 70% sensitivity, 42% specificity and poor agreement (κ = 0.113). The negative control serum was reported negative by PAX and MAC, whereas positive reactions of the negative control contributed to the lower specificity of Allercept. Quantitative intra-assay reproducibility was assessed using all four IgE values from the serum mixes with identical low concentrations of specific IgE to the same allergen. The PAX showed low variability (1.8% to 7.4%), whereas both the MAC and Allercept had significantly higher variations of 144.2 % and 200% respectively, indicating a poor reproducibility.

Interpretation and Clinical Relevance

These findings indicate that SAT’s are not analytically interchangeable. False-negative results may lead to omission of relevant allergens from AIT, whereas false-positive results may lead to inclusion of irrelevant allergens. The superior performance of the molecular allergology-based test (PAX) may reflect its use of defined allergen components rather than crude extracts. Variation in allergen extract composition and low concentrations of relevant components likely contributed to the reduced sensitivity in the extracts based assays (MAC and Allercept).

Conclusion

Using sera with known monoclonal IgE composition, the molecular allergology-based PAX demonstrated the highest analytical accuracy. The MAC and Allercept showed lower agreement with the expected IgE profiles and markedly higher variability, particularly at low IgE concentrations. These findings suggest that methodology plays a critical role in the detection and quantification of allergen-specific IgE and should be considered when interpreting SAT results.

Strengths and Limitations

A major strength of this study is the use of sera with predefined allergen-specific dog IgE composition, allowing direct accuracy assessment in a way that is not possible with naturally sensitized canine sera. The samples were randomized and sent, coded, to the external investigator who submitted them with random patient identification, thereby reducing the risk of interpretation bias. However, artificial sera might not fully reproduce the biological complexity of naturally sensitized canine sera, and further studies are needed to determine how these results translate into clinical practice.

Funding and Conflicts of Interest

Source of funding: Nextmune AB, Stockholm, Sweden; the Danish Veterinary Dermatology Association; and Vetgruppen Birkerød Dyrehospital, Denmark. The sponsors were not involved in the calculation of the comparative performance characteristics. Conflicts of interest: The tested material was developed and commercialized by Nextmune. No other conflicts declared.