

Research Article

Proficiency Monitoring of Allergen-Specific IgE *macELISA* - 2021

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Abstract

The purpose of this study was to evaluate the reproducibility of results yielded using a *macELISA* for detection of allergen specific IgE in dogs and cats when run by eleven different individuals in seven separate affiliated laboratories. Samples of 24 different sera samples were independently evaluated in each laboratory by differing operators in a single blinded fashion. For evaluations completed by multiple operators in a single laboratory, the average intra-operator variance was calculated to be 4.6% (range=0.8%-8.7%) while the average inter-operator variance was 5.7% (range=1.4%-7.8%). The average intra-assay variance among reactive assay calibrators in all laboratories was 5.3% (range=0.8%-12.6%). The overall inter-assay inter-laboratory variance evident with reactive calibrators was consistent among laboratories and averaged 10.1% (range=4.4%-12.8%). All laboratories yielded similar profiles and magnitudes of responses for replicate unknown samples; dose response profiles observed in each of the laboratories were indistinguishable. Correlation of EAU observed for individual allergens between and among all laboratories was strong ($r > 0.90$, $p < 0.001$). Collectively, the results demonstrated that ELISA for measuring allergen specific IgE is reproducible, and documents that consistency of results can be achieved not only in an individual laboratory, but among different operators and between laboratories using the same ELISA.

Keywords: IgE, ELISA, Proficiency, Atopy, Allergy, Immunotherapy, Cross-reactive carbohydrate

Introduction

Stallergenes Greer maintains a proficiency monitoring program for laboratories that routinely run *macELISA* [1] for evaluation of allergen specific IgE in serum samples. The foundation for this program is based on the desire for inter-laboratory standardization and quality control measures that ensure the uniformity, consistency, and reproducibility of results among laboratories that perform the assays. This program is designed to evaluate the proficiency of laboratories and ensures that individual operators yield consistent and reproducible results. The inaugural proficiency evaluations, initiated in 2009 and repeated in 2010, in six different laboratories documents that inter-laboratory standardization and quality control measures in the veterinary arena are on the immediate forefront and that uniformity, consistency, and reproducibility of results between laboratories is achievable [2]. Similarly, reproducibility of results among different laboratories was documented in the subsequent proficiency evaluations completed in 2013 [3], 2016 [4], 2018 [5], 2019 [6], and 2020 [7]. The results presented herein summarize the comparative results observed in the affiliate laboratories for the most recent proficiency evaluations that were completed in August 2021.

The 2021 proficiency evaluation is the third documentation of the assay reproducibility since adopting a cross-reactive carbohydrate inhibitor in the sample diluent [7,8].

Materials and Methods

All serum samples, buffers, coated wells, calibrator solutions, and other assay components were aliquants of the respective lots of materials manufactured at Stallergenes Greer's production facilities (located in Lenoir, NC, USA) and supplied as complete kits to the participating laboratories along with the exact instructions for completing the evaluations.

Participating Laboratories

Seven independent Veterinary Reference Laboratories (VRLs) participated in the 2021 proficiency evaluation of *macELISA*. Participating laboratories included three separate IDEXX laboratories located in Memphis, Tennessee, Kornwestheim, Germany, and Markham, Ontario Canada. Other affiliated European laboratories that participated in this evaluation included Agrolabo (Scarmagno, Italy), Laboratories LETI Pharma (Barcelona, Spain), and Ceva

Biovac (Beaucouzé, France). Stallergenes Greer Laboratories (Lenoir, NC) served as the prototype for evaluation of the *macELISA*; the 2021 evaluations included results reported by three separate and independent operators. Because the performance characteristics of *macELISA* in Stallergenes Greer's VRL have been well documented for use over an extended period [1-6], all results observed in the other participating laboratories were compared directly with the results observed in Stallergenes Greer's reference laboratory.

Serum Samples

Separate pollen and mite reactive serum pools or non-reactive sera pools were prepared from cat and dog serum samples that previously had been evaluated using the *macELISA* for detection of allergen specific IgE. The reactivity of each sera pool ranged from nonreactive to reactive for multiple pollen or mite allergens. These sera pools and admixtures of the pools were used to construct a specific group of samples that exhibited varying reactivity to the allergens included in the evaluation panel. Twenty-four samples were included in the blinded evaluation conducted by each laboratory. Identical replicates of the high, low, and negative controls routinely used in the assay were also included as unknown samples. Also included in the array of samples was a five tube three-fold serial dilution of a highly pollen reactive pool, diluted into non-reactive sera, which served to document the dose response evident within the assay. All samples were stored at -20°C for the interim between testing.

Calibrators

Mite reactive calibrator solutions of predetermined reactivity in the *macELISA* were prepared as three-fold serial dilutions of a sera pool highly reactive to *Dermatophagoides farinae*, *Acarus siro*, and *Tyrophagus putrescentiae*. Replicates of each were evaluated in each assay run and served as a standard response curve for normalizing results observed with the various samples. All results were expressed as ELISA Absorbance Units (EAU) which are background-corrected observed responses expressed as milli absorbance.

Buffers

The buffers used throughout have been previously described [1-7] and included: a) well coating buffer: 0.05 M sodium carbonate bicarbonate buffer, pH 9.6; b) wash buffer: phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20, and 0.05% sodium azide; c) reagent diluent buffer: PBS, pH 7.4, containing 1% fish gelatin, 0.05% Tween 20 and 0.05% sodium azide. The buffer used for dilution of serum samples was identical to the reagent diluent buffer, but it has been supplemented (2.5 mg/mL) with a cross-reactive carbohydrate inhibitor derived from the carbohydrate components present in bromelain (BROM-CCD)7. BROM-CCD was prepared in house and remains a proprietary product of Stallergenes Greer (Lenoir, NC, USA).

Allergen Panel

The allergen panel was a 24 allergen composite derived from the array of allergens that are included in the specific panels routinely evaluated in the various laboratories; the composite allergen panel

consisted of 4 grasses, 6 weeds, 6 trees, 5 mites, and 3 fungi. The protocol for coating and storage of wells has been previously described [1-7]. Immulon 4HBH flat bottom 12 well strip assemblies (Thermo Electron Corporation, Waltham, MA) were used throughout and served as the solid phase for all assays. The individual extracts were diluted in bicarbonate buffer (pH 9.6) and 100 µL was added to each assigned well. Following overnight incubation at 4-8°C, the wells were washed with PBS, blocked with 1% monoethanolamine (pH 7.5) then air dried and stored at 4-8°C in Ziploc bags until used.

Sample Evaluations - *macELISA*

The operational characteristics and procedures for the *macELISA*s have been previously described [1-6]. Following incubation of allergen coated wells with an appropriately diluted serum sample, allergen-specific IgE is detected using a secondary antibody mixture of biotinylated monoclonal anti-IgE antibodies, streptavidin alkaline phosphatase as the enzyme conjugate, and p-nitrophenylphosphate (pNPP) as substrate reagent. Specific IgE reactivity to the allergens is then estimated by determining the absorbance of each well measured at 405 nM using an automated plate reader. All results are expressed as ELISA Absorbance Units (EAU), which are background-corrected observed responses expressed as milli absorbance [8-13].

To evaluate the stability of stored wells, the reactivity of wells coated in April 2019 and in April 2021 was assessed by Stallergenes Greer technicians. The storage stability of the anti-IgE-biotin reagent was assessed by comparing the reactivity of a preparation of reagent that was prepared in January 2017 with that of one that was prepared in April 2020.

Statistics

A coefficient of variation was calculated as the ratio of standard deviation and means of the responses observed for the calibrator solutions within different runs in multiple laboratories. Pearson's correlation statistic was used for inter-laboratory comparison among individual allergens. Statistical analyses were conducted using EXCEL (2016; Microsoft; Redmond, WA, USA).

Results

The assay variance (% CV) observed with the calibrator solutions in the different laboratories are presented in Table 1 and are representative of the assay reproducibility in the various laboratories. The average intra-assay % CV among positive calibrators (#1-5) was 5.3% (range=0.8%-12.6%); differences among laboratories or between assays and within assay runs were not detected. No substantial difference in results among various operators was revealed. The average intra-operator variance documented for Stallergenes Greer technicians was calculated to be 4.6% (range=0.8%-8.7%) while the average inter-operator variance was 5.7% (range=1.4%-7.8%). The average inter-assay variance (% CV) observed in Stallergenes Greer's laboratory with the positive calibrators from multiple runs over a one year period has been documented at 8.9% (range 7.1%-9.7%), and the inter-laboratory % CV among reactive calibrators also remained

relatively constant (average 12.1%; range=11.2%-13.4%). The results of the current evaluation (Table 1) are consistent with these unpublished findings; the inter-assay variance among positive calibrators for all laboratories included in this evaluation was calculated to be 10.1% (range=4.4%-12.8%). Similar to previously published studies [1-7], the intra-assay variability was higher with the calibrators containing lesser amounts of allergen specific IgE, and a similar increased intra-assay variability was evident with the background ODs (average 8.6%; range=2.0%-18.1%). A negative response is classified as anything with an EAU below 150 [1]. Any analysis of results below this threshold, especially when looking at %CV and relative differences should be done cautiously.

To evaluate the strength of association with the magnitude of EAU results observed for each allergen among the different laboratories a Pearson's correlation coefficient was determined (Microsoft Excel 2016) for each laboratory pair. Because the *mac*ELISA is designed to yield comparable responses in dog and cat samples, comparison of results among affiliate laboratories included both cat and dog samples as a single population of sera samples [5-7]. These results (Table 2) demonstrate that very high inter-laboratory correlation ($r>0.90$; $p<0.001$) is evident between the results observed in Stallergenes Greer laboratory and those observed in six affiliate laboratories for all pollen, mite, and fungi allergens. The overall correlation of results observed in the various laboratories is summarized in Table 3; a very strong correlation ($r>0.90$, $p<0.001$) was demonstrated between and among the results of the participating laboratories.

For an evaluation of the dose response in this ELISA, a five tube three-fold serial dilution of a reactive dog sera pool was prepared using a negative cat sera pool as diluent. Each of the dilutions was then evaluated by all of the participating laboratories as unknown independent samples. Similar responses were yielded by all of the laboratories and the results observed within the various laboratories are encompassed by the acceptable variance limits [1-3] ($\pm 20\%$) established for *mac*ELISA. Further, the magnitude of responses observed in each laboratory was reduced in direct proportion to dilution. Consequently, the dose responses for the individual allergens are presented as aggregate responses. The results presented in Figure 1 confirm the sera pool was highly reactive to mites as well as grass, weed, and tree pollen allergens, but it was not reactive to fungi. To be expected, the magnitude of responses observed in each laboratory was reduced in direct proportion to dilution. Results from the final tube in the dilution scheme yielded results that were indistinguishable from negative responses, indicating a dilution extinction of detectable response.

The final objective for the current evaluation was to document the stability of the anti-IgE biotin reagent and the allergen coated wells. For these evaluations two separate lots of each assay component were reciprocally assessed. The two lots of allergen coated wells were manufactured in April 2019 and April 2021 and stored in zip closure plastic bags at 4-8°C until used. The separate anti-IgE reagent lots were manufactured in January 2017 and April 2021 and were stored at -10°C

in alkaline phosphatase stabilizing buffer containing 50% glycerin. All evaluations were completed in July 2021.

The results present in Table 4 demonstrate that similar responses are yielded with calibrators when evaluated with either lot of anti-IgE biotin. The average intra assay variance (% CV) observed with the two reagent lots were evaluated by two separate technicians and calculated to be 5.5% (range=0.9%-11.9%). The average inter-assay variance for the two reagent lots was calculated to be 7.1% (range=1.1%-12.0%); whereas the average inter-lot/inter-operator variance was 12.1% (range=2.2%-17.9%). To be expected, the greatest variance was noted for calibrator solutions that yielded signals of lesser magnitude.

The final endeavor of the present study was to document the storage stability of the individual allergen coated wells along with the anti-IgE biotin conjugate stability. For these evaluations a single dilution of each of the sera included in the proficiency panel were evaluated separately by two technicians on both lots of wells using each of the anti-IgE biotin lots. Because the magnitude of signals evident with the individual allergens between the two technicians was indistinguishable all results were treated as a single population for each allergen. The results present in Table 5 demonstrate a very high correlation (Pearson's) of results for each of the allergen coated wells and for each lot of anti-IgE biotin ($r>0.900$, $p<0.001$).

Discussion

Consistent with previous proficiency evaluations of laboratories that routinely run the monoclonal antibody cocktail based enzyme linked immunoassay (*mac*ELISA) manufactured by Stallergenes Greer [1-6], the results of the present study demonstrated that the intra-assay variance observed with the positive calibrators remains relatively low and indistinguishable among the various laboratories. Likewise, the inter-assay variance within each laboratory remained relatively constant and the results from all laboratories was demonstrably similar and the CV of the positive responses was relatively constant across the entire range of reactivity tested. The results demonstrated that the variability between and among the affiliate laboratories and technicians are indistinguishable from the results evident within and between runs completed in the laboratory of Stallergenes Greer. Thus, all laboratories and technicians included in the study were equally proficient in providing consistent results for all allergens tested and the results were well within the acceptable variance limits ($\pm 20\%$) established for this assay and reflects the robustness of the assay [1].

During the 2020 proficiency evaluations, we documented the stability of allergen specific IgE in serum samples stored frozen for at least one year. In the present study we have documented the stability of allergen coated wells that have been stored for at least 24 months. In addition, we have shown that our anti-IgE reagent maintain functional utility for at least 55 months when stored at -10°C in an alkaline phosphatase stabilized buffer containing 50% glycerin.

There was no compelling evidence that the level of allergen specific IgE correlates with severity of clinical disease [14-17]. However, an evaluation that purports to measure allergen specific IgE should have a reduction in signal that is directly proportional to the dilution factor of the test ligand [18]. Similar responses were yielded by all of the laboratories for the samples that comprised the dose response and the results observed within the various laboratories are encompassed by the acceptable variance limits ($\pm 20\%$) established for macELISA [1-3]. Further, the magnitude of responses observed in each laboratory was reduced in direct proportion to dilution. Consequently, the dose responses for the individual allergens are presented as aggregate responses (Figure 1). The responses of greatest magnitude were evident with the grass pollen allergens, and these responses were reduced in direct proportion to dilution; the magnitude of responses ranged from near maximum to those that were indistinguishable from background responses. The reaction profiles for grass allergens also appear to be parallel and quite similar in magnitude of response. Whether or not these like responses result because of a similar level of co-sensitization or allergen epitope similarity combined with cross-reaction remains to be determined. Although the responses evident to differing tree and weed allergens are more variable in magnitude of response, the observed response in each laboratory was reduced in direct proportion to dilution. The positive response profiles evident with these allergens also appear to be parallel and, it becomes evident that the detectability of allergen specific IgE within this assay spans at least a 150-fold dilution range. Substantial responses to *A siro*, *D farinae*, and *T putrescentiae* were

noted in the original sample and these responses decreased in direct proportion to dilution. Reactivity to fungal allergens were lacking in the original sample. We have demonstrated a continued reliability and reproducibility of our macELISA with the open publication of our proficiency testing procedures and results [1-6]. We encourage other groups to determine and document similar findings; however, we emphasize the importance of identifying results below the cutoff of 150 EAU merely as non-reactive and consequently negative responses. The reproducibility of the assay for these responses should be defined only as negative and their numerical values become meaningless; comparison of EAU values are meaningful for reactive samples only (EAU > 150). Because the magnitude of specific responses is dependent on the concentration of allergen-specific IgE that can span a wide range, a better means of comparison of repeat results for individual samples in an assay of this sort should be to evaluate the correlation (perhaps Pearson statistic) of results that might exist.

The lack of a regulatory mandated quality assurance program for serum allergen specific IgE testing in companion animals, that independently monitors performance of all laboratories and assay formats, prompts Stallergenes Greer to focus on its continued evaluation of laboratories that routinely use the company's assays. Information presented herein documents the continued commitment of Stallergenes Greer and its affiliate laboratories to providing a stream of information relating these results to the veterinary community.

Table 1: Calculated variance of macELISA calibrator solutions observed with different laboratory runs by multiple operators during the 2021 Proficiency evaluation.

Variance	Calibrator % CV*						BG†
	N	#1	#2	#3	#4	#5	% CV
Inter-Laboratory	352	4.4	10.0	12.8	12.2	10.8	19.8
Inter-Assay (<i>Stallergenes Greer</i>)	160	1.4	6.2	7.8	6.7	6.6	7.7
Intra-Assay							
Stallergenes Greer #1	32	1.3	6.1	5.9	4.5	3.7	3.8
Stallergenes Greer #2	32	1.2	3.4	6.8	4.5	6.5	2.0
Stallergenes Greer #3	32	1.3	6.7	7.9	4.8	6.1	9.9
Stallergenes Greer #4	32	0.8	4.1	4.2	5.0	3.9	4.9
Stallergenes Greer #5	32	1.1	4.7	4.6	8.7	7.0	7.9
IDEXX Memphis	32	3.3	6.9	12.2	7.0	10.9	14.6
IDEXX Canada	32	1.5	3.0	5.2	4.2	3.5	9.4
IDEXX Germany	32	2.0	6.6	11.1	7.5	4.0	9.9
Agrolabo	32	1.0	3.2	5.7	6.9	12.6	18.1
Biovac	32	2.6	7.2	6.6	6.3	6.8	6.0
LETI	32	2.2	4.5	8.9	6.7	7.3	7.6

*Calibrator #1 was prepared as a dilution of a sera pool which is highly reactive to mite allergens; Calibrators #2 - #5 are prepared as a serial 3-fold dilution of calibrator #1.

†Background responses observed with diluent in place of serum sample.

Table 2: Inter-laboratory correlation of macELISA results observed with individual allergens in Stallergenes Greer Laboratory and the results observed in the individual affiliate laboratories.

Allergens	Inter-Laboratory Coefficient of Correlation* Stallergenes Greer vs					
	IDEXX Memphis	IDEXX Germany	IDEXX Canada	Ceva Biovac	Agrolabo	LETI
Mites						
Acaris siro	0.996	0.995	0.981	0.988	0.995	0.972
Dematophagoides farinae	0.992	0.993	0.998	0.984	0.994	0.972
Dematophagoides pteronyssinus	0.990	0.982	0.971	0.980	0.976	0.882
Lepidoglyphus destructor	0.971	0.967	0.893	0.953	0.974	0.838
Tyrophagus putrescentiae	0.993	0.986	0.975	0.992	0.991	0.956
Grasses						
June Grass (<i>Poa pratensis</i>)	0.995	0.990	0.997	0.988	0.990	0.937
Meadow fescue (<i>Festuca pratensis</i>)	0.989	0.987	0.983	0.983	0.987	0.908
Orchard Grass (<i>Dactylis glomerata</i>)	0.984	0.982	0.993	0.984	0.984	0.904
Perennial Rye (<i>Lolium perenne</i>)	0.985	0.984	0.988	0.983	0.986	0.899
Trees						
Birch (<i>Betula pendula</i>)	0.977	0.978	0.965	0.908	0.964	0.900
Cypress (<i>Cupressus sempervirens</i>)	0.981	0.972	0.904	0.948	0.898	0.926
Hazelnut (<i>Corylus avellana</i>)	0.978	0.970	0.983	0.977	0.955	0.915
Olive (<i>Olea europaea</i>)	0.980	0.972	0.991	0.964	0.965	0.889
Populus mix (<i>P. nigra</i> , <i>P. tremula</i> , <i>P. alba</i>)	0.976	0.970	0.988	0.975	0.964	0.894
Willow Black (<i>Salix discolor</i>)	0.971	0.962	0.963	0.976	0.968	0.927
Weeds						
English Plantain (<i>Plantago lanceolata</i>)	0.988	0.974	0.979	0.985	0.977	0.905
Lambs Quarter (<i>Chenopodium album</i>)	0.981	0.961	0.944	0.933	0.934	0.916
Mugwort (<i>Artemisia vulgaris</i>)	0.977	0.967	0.980	0.971	0.953	0.915
Pellitory (<i>Parietaria officinalis</i>)	0.985	0.974	0.986	0.981	0.977	0.914
Ragweed (<i>Ambrosia trifida</i> , <i>A. artemisiifolia</i>)	0.999	0.992	0.996	0.997	0.989	0.994
Sheep Sorrel (<i>Rumex acetosella</i>)	0.990	0.982	0.986	0.988	0.980	0.931
Fungi						
<i>Alternaria alternata</i>	0.953	0.991	0.977	0.977	0.945	0.931
<i>Aspergillus fumigatus</i>	0.993	0.995	0.994	0.958	0.998	0.950
<i>Cladosporium herbarum</i>	0.995	0.992	0.843	0.914	0.994	0.944
Overall	0.991	0.986	0.977	0.987	0.983	0.956

*Pearson Correlation Coefficient (r); Good Correlation ($r > 0.8$, $p < 0.001$).**Table 3:** Inter-laboratory correlation of macELISA results observed among individual affiliate laboratories.

Laboratory	Interlaboratory Coefficient of Correlation*						
	Stallergenes Greer	IDEXX Memphis	IDEXX Germany	IDEXX Canada	Ceva Biovac	Agrolabo	LETI
Stallergenes Greer	1	0.991	0.986	0.977	0.987	0.983	0.956
IDEXX Memphis	0.991	1	0.990	0.980	0.989	0.986	0.967
IDEXX Germany	0.986	0.990	1	0.967	0.981	0.994	0.956
IDEXX Canada	0.977	0.980	0.967	1	0.978	0.962	0.955
Biovac	0.987	0.989	0.981	0.978	1	0.974	0.969
Agrolabo	0.983	0.986	0.994	0.962	0.974	1	0.948
LETI	0.956	0.967	0.956	0.955	0.969	0.948	1

*Pearson Correlation Coefficient (r); Good Correlation ($r > 0.8$, $p < 0.001$).

Table 4: Correlation of responses observed with calibrator solutions when evaluated with separate lots of anti-IgE biotin that were stored for 3 or 55 months.

Variance	Anti-IgE Biotin		N	#1	Calibrator % CV*				
	Lot #	Storage [†] (Months)			#2	#3	#4	#5	BG % CV
Inter-Lot/Operator	1 & 2	3 & 55	160	2.2	9.8	15.7	17.9	15.0	9.5
Inter-Assay									
	1	55	96	1.5	7.6	6.3	7.0	7.8	7.0
	2	3	96	1.1	5.7	10.6	12.0	11.2	10.3
Intra-Assay									
Operator #1	1	55	64	1.6	9.0	4.9	4.0	3.1	4.0
	2	3	64	0.9	3.2	6.7	5.5	7.4	3.8
Operator #2	1	55	32	1.2	3.4	6.8	4.5	6.5	6.8
	2	3	32	1.2	6.3	10.1	11.9	11.5	11.4

*Calibrator #1 was prepared adilution of a sera pool which is highly reactive to mite allergens; Calibrators #2 - #5 are prepared as a serial 3-fold dilution of calibrator #1. Background responses observed with diluent in place of serum sample.

[†]Anti-IgE biotin was stored at -10°C in alkaline phosphatase stabilizing buffer containing 50% glycerin.

Table 5: Correlation of results observed with proficiency sera samples when evaluated with two separate lots of anti-IgE biotin stored for 3 or 55 months using allergen coated wells that were stored for 3 and 24 months.

Allergens	Coefficient of Correlation			
	Wells* Apr 2019 vs. Apr 2021		Biotin [†] Jan 2017 vs. Apr 2020	
	Biotin Jan 2017	Biotin Apr 2020	Wells Apr 2019	Wells Apr 2021
Mites				
Ascaris siro	0.993	0.992	0.995	0.994
Dermatophagoides farinae	0.999	0.999	0.996	0.993
Dermatophagoides pteronyssinus	0.987	0.985	0.995	0.988
Lepidoglyphus destructor	0.959	0.975	0.987	0.976
Tyrophagus putrescentiae	0.988	0.987	0.996	0.991
Grasses				
June Grass (<i>Poa pratensis</i>)	0.995	0.997	0.965	0.946
Meadow fescue (<i>Festuca pratensis</i>)	0.998	0.998	0.956	0.937
Orchard Grass (<i>Dactylis glomerata</i>)	0.990	0.996	0.952	0.926
Perennial Rye (<i>Lolium perenne</i>)	0.993	0.997	0.956	0.929
Trees				
Birch (<i>Betula pendula</i>)	0.997	0.971	0.903	0.960
Cypress (<i>Cupressus sempervirens</i>)	0.874	0.918	0.956	0.962
Hazelnut (<i>Corylus avellana</i>)	0.998	0.998	0.944	0.947
Olive (<i>Olea europaea</i>)	0.999	0.995	0.935	0.950
Populus mix (<i>P. nigra</i> , <i>P. tremula</i> , <i>P. alba</i>)	0.995	0.992	0.942	0.949
Willow Black (<i>Salix discolor</i>)	0.983	0.973	0.931	0.953
Weeds				
English Plantain (<i>Plantago lanceolata</i>)	0.995	0.985	0.957	0.982
Lambs Quarter (<i>Chenopodium album</i>)	0.996	0.992	0.952	0.962
Mugwort (<i>Artemisia vulgaris</i>)	0.980	0.985	0.954	0.950
Pellitory (<i>Parietaria officinalis</i>)	0.997	0.996	0.940	0.953
Ragweed (<i>Ambrosia trifida</i> , <i>A. artemisifolia</i>)	0.999	0.990	0.997	0.992
Sheep Sorrel (<i>Rumex acetosella</i>)	0.999	0.991	0.942	0.963
Fungi				
<i>Alternaria alternata</i>	0.989	0.993	0.986	0.969
<i>Aspergillus fumigatis</i>	0.997	0.998	0.996	0.993
<i>Cladosporium herbarum</i>	0.932	0.923	0.974	0.966
Overall	0.972	0.975	0.974	0.975

*Allergen coated wells were air dried then stored at 4-8°C in plastic bags.

[†]Anti-IgE biotin was stored at -10°C in alkaline phosphatase stabilizing buffer containing 50% glycerin.

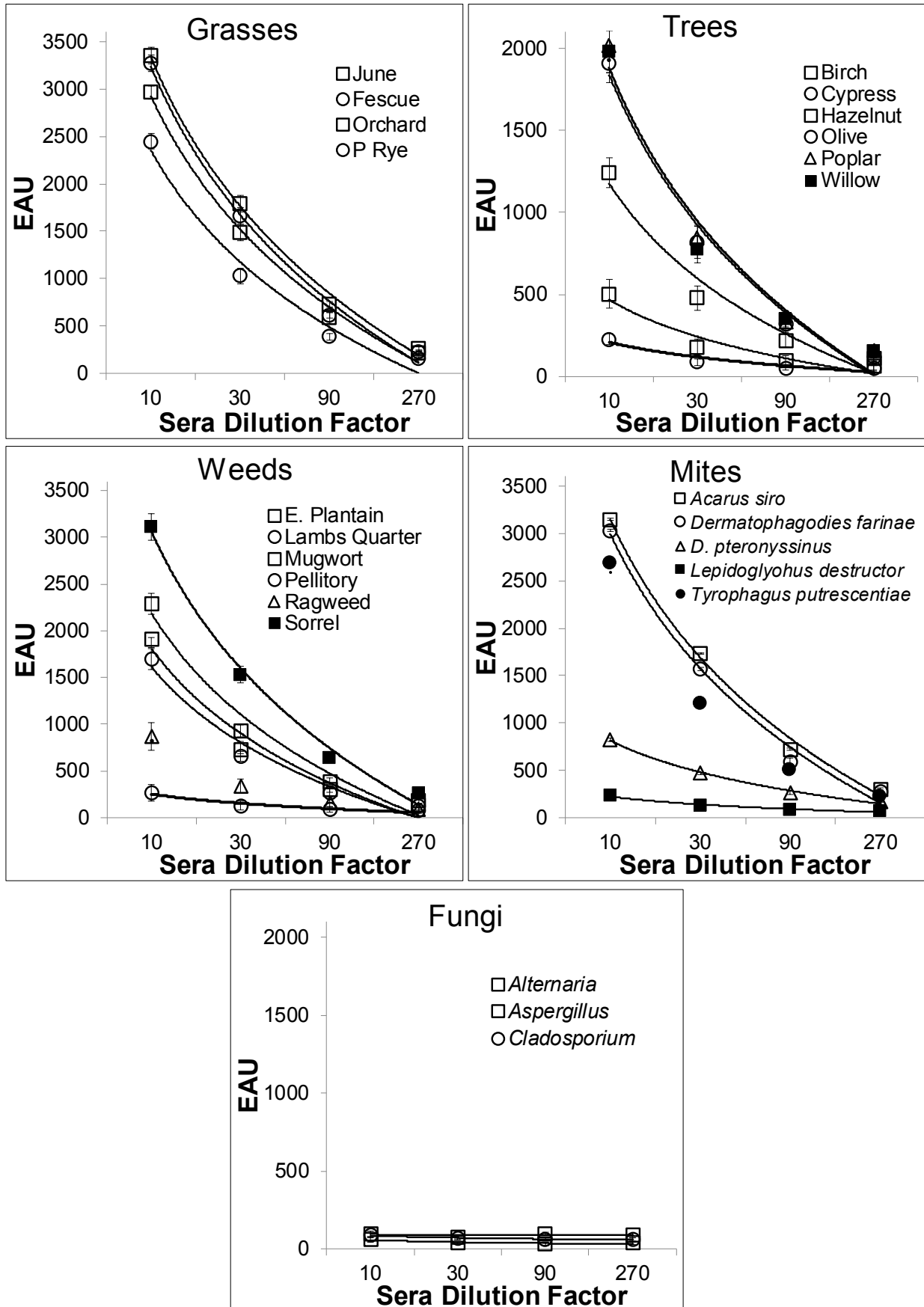


Figure 1: Dose response evident in *macELISA* with a pollen reactive serum pool.

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