

Highly sensitive ELISA-based assay for quantification of allergen-specific IgE antibody levels

To the Editor:

Allergy is the most common immunologically mediated hypersensitivity disease worldwide affecting upper and lower respiratory tract, the skin as well as the gastrointestinal tract and may cause life-threatening anaphylaxis.¹ Immunoglobulin E (IgE) occurs in extremely low amounts in blood and tissues but is the key immunoglobulin in allergy. The quantification of allergen-specific IgE levels (sIgE) in serum and body fluids is important because sIgE concentrations are useful to define clinical phenotypes of allergy and to predict the intensity of allergic reactions.²⁻⁵ However, quantification of sIgE requires special instruments (eg, ImmunoCAP) that are available only in industrialized countries, large volumes of serum and is quite expensive.⁶ Therefore, the goal of our study was to establish a quantitative method for measuring allergen-specific IgE based on a simple ELISA technology which can be implemented and used in all parts of the world. The user will thus need only equipment for ELISA, reagents, detection antibodies and test allergens plus reagents for the IgE standard curve.

In order to establish a standard for ELISA-based quantification of allergen-specific human IgE, a human monoclonal chimeric IgE antibody (IgEmoAb)⁷ consisting of the variable region of a mouse IgG₁ antibody specific for the major birch pollen allergen Bet v 1, which had been fused to the human epsilon heavy chain, was expressed and purified from hybridoma cells by affinity chromatography to >95% purity (Figure S1A). Under non-reducing conditions, purified IgEmoAb migrates as a distinct single band of approximately 250 kDa molecular weight in SDS-PAGE, while under reducing conditions, two distinct bands which correspond to the heavy and light chain became visible at approximately 100 and 20 kDa, respectively (Figure S1A). IgEmoAb reacted specifically with anti-human IgE antibodies under non-reducing conditions, and under reducing conditions, the heavy chain showed selective anti-IgE reactivity by immunoblotting (Figure S1B). Structural analysis of IgEmoAb by circular dichroism showed that it assumed a predominant beta-sheet fold with a minimum signal around 217 nm and a maximum near 198 nm (Figure S1C).

Using IgEmoAb and Bet v 1, we next established by ELISA a standard curve allowing to measure and quantify sIgE. A series of dilutions of IgEmoAb ranging from 2.42 to 2420 ng/mL was exposed to ELISA plate-bound IgE yielding standard curves with a linear

range of approximately 2.42-242 ng/mL IgE depending on the time of assay development (Figure 1A). Figure S2 shows the OD values obtained for the dilution series of IgEmoAb determined by ELISA at different time points of assay development, that is, (after 5, 10, 15, and 20 minutes) yielding optimal results from 5 to 10 minutes of development with the given detection antibodies and color reagents. The different dilutions of IgEmoAb were also measured using Streptavidin ImmunoCAPs prepared with the same recombinant Bet v 1 preparation used in the ELISA experiments. The concentrations of IgEmoAb, that is, 2.42, 24.2, 242, and 2420 ng/mL corresponded to the following ImmunoCAP results: 0.34, 3.28, 33.1, and >100 kU_A/L showing that the dilutions of the IgEmoAb standard were correct down to few nanograms per mL without loss of activity. However, we noted that the ImmunoCAP determination of the concentrations of IgEmoAb was different (ie, yielding approx. 30%) from the results obtained by microBCA protein determination which may be due to differences between the ImmunoCAP IgE standard and IgEmoAB (data not shown). Accordingly, a "real conversion factor" of 7 ng/mL = 1 kU_A/L for calculating IgE concentrations had to be introduced.

Based on a standard curve established with IgEmoAb, Bet v 1-specific IgE concentrations were quantified in 19 sera from clinically well-characterized birch pollen allergic patients by ELISA (ng/mL) (Tables S1 and S2). Table S2 displays the OD levels, the corresponding ng/mL concentrations of Bet v 1-specific IgE, and the results from the ImmunoCAP and ImmunoCAP ISAC measurements in detail. We noted a strong significant correlation between the Bet v 1-specific IgE concentrations determined in ng/mL with the ELISA and in kU_A/L ImmunoCAP results ($r = .904$, $P < .0001$, 95% CI 0.756-0.964; Figure 1B). In fact, Bet v 1-specific IgE levels measured by ELISA correlated even better with ImmunoCAP results than IgE levels measured with ImmunoCAP ISAC chip technology ($r = 0.875$, $P < .0001$, 95% CI 0.692-0.953; Figure 1C). Nevertheless, results of the quantitative IgE ELISA correlated also significantly with sIgE levels measured with ImmunoCAP ISAC chip technology ($r = .840$, $P < .0001$, 95% CI 0.616-0.939) (Figure 1D). In summary, strong correlations of Bet v 1-specific IgE levels measured by ELISA with those determined by ImmunoCAP and ImmunoCAP ISAC technology were obtained.

Next, we investigated whether the IgEmoAb-based standard curve is also suitable for the quantification of patients' IgE to an

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allergen molecule unrelated to Bet v 1. For this purpose, we selected the major cat allergen Fel d 1 which besides Bet v 1 is one of the most frequently recognized allergens in the Russian population.^{5,8} Quantitative IgE ELISA was used to determine Fel d 1-specific IgE levels in 19 cat allergic patients using different serum dilutions. Detailed results of quantitative IgE ELISA and of ImmunoCAP are shown in Tables S3 and S4. A strong significant correlation was obtained between Fel d 1-specific IgE concentrations quantified with the IgE ELISA (ng/mL) and with the ImmunoCAP results (kU_A/L) ($r = .984$, $P < .0001$, 95% CI 0.956-0.994; Figure 2A) demonstrating that the quantitative ELISA can be used for quantification of specific IgE levels against different allergens, for example, Fel d 1. Interestingly, we noted for three patients with clinically confirmed symptoms of cat allergy ImmunoCAP measurements were below the threshold of 0.1 kU_A/L but we were able to detect and quantify Fel d 1-specific IgE with the IgE ELISA (Table S3, patients C4, C5, and C12). Of note, we found that the results obtained for different

dilutions of the sera delivered highly comparable results. Results of IgE level assessment obtained from 1:2 sera dilutions and 1:4 sera dilutions from the cat allergic patients (shown in Table S4) were compared, and a strong and significant correlation was noted ($r = .988$, $P < .0001$, 95% CI 0.967-0.996; Figure 2B).

The quantitative IgE ELISA assay should be useful for laboratories in less developed countries which cannot afford expensive IgE test systems for routine allergy diagnosis taking advantage of molecular allergy diagnosis.^{9,10} Before extensive use, broader clinical studies correlating IgE concentrations to clinical signs may be warranted. In fact, the price for a quantitative IgE determination for one allergen should cost less than 10% than by ImmunoCAP; however, the time required for testing will be longer. Our assay will therefore be useful in places where work power cost is not a major issue and the results are desired in a higher-throughput format as well as for cases (eg, research) where regulations are of minor importance. Furthermore, the assay should allow a

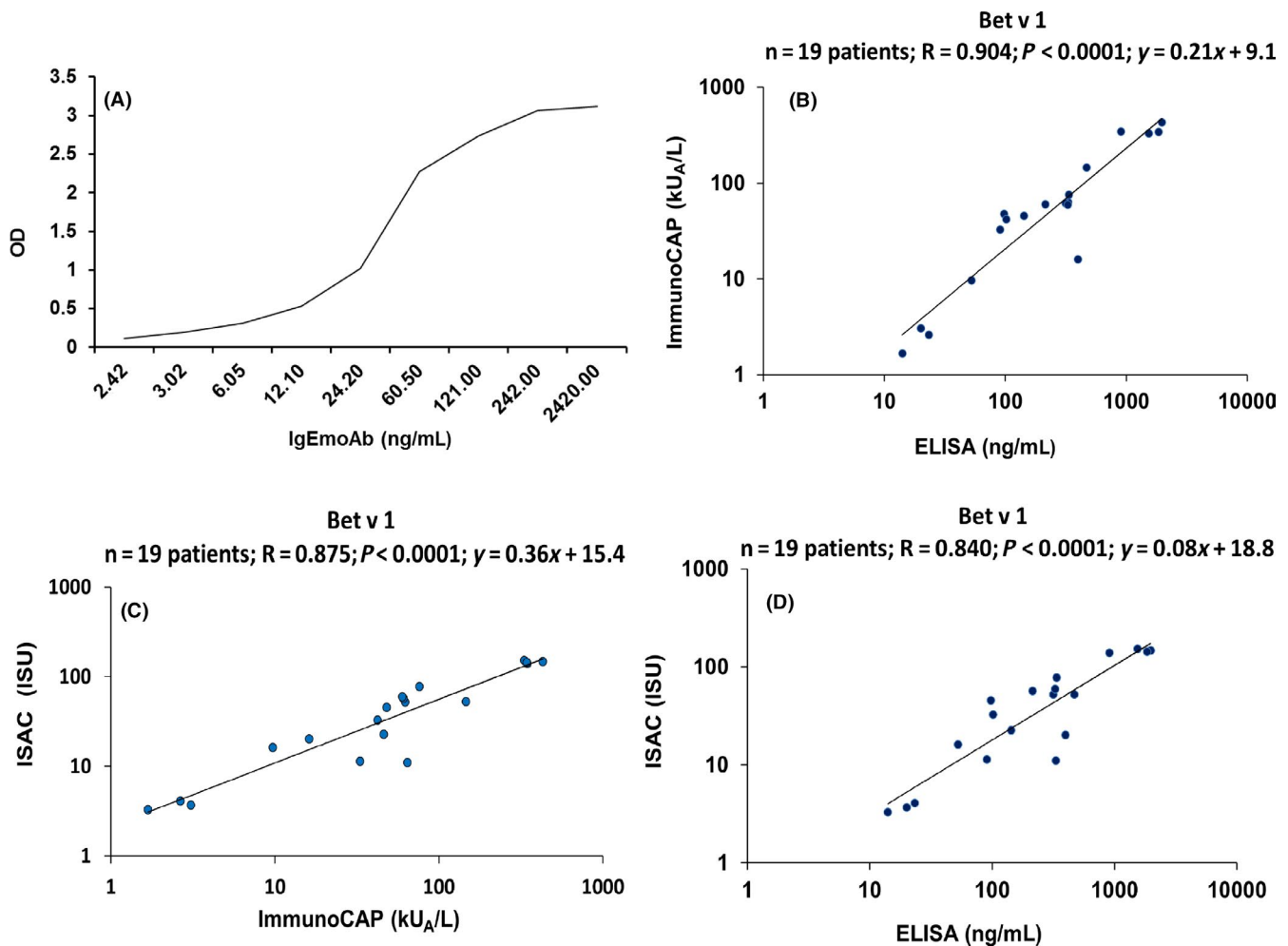


FIGURE 1 Association of different concentrations of IgE (x-axis: ng/mL) with IgE reactivities to Bet v 1 determined as optical density (OD) values (y-axis) by ELISA (A). Correlation of Bet v 1-specific IgE levels determined in sera from 19 birch pollen allergic patients by different methods. B, ImmunoCAP (kU_A/L) (y-axis) versus ng/mL determined by ELISA (x-axis). C, ISU (ISAC standardized unit) values determined by ImmunoCAP ISAC (y-axis) vs ImmunoCAP (kU_A/L) (x-axis) and D, ISU values determined by ImmunoCAP ISAC (y-axis) vs ng/mL determined by ELISA (x-axis). Correlation coefficients (r), regression equations, and statistical significances (P values) are shown in the top upper corner of graph

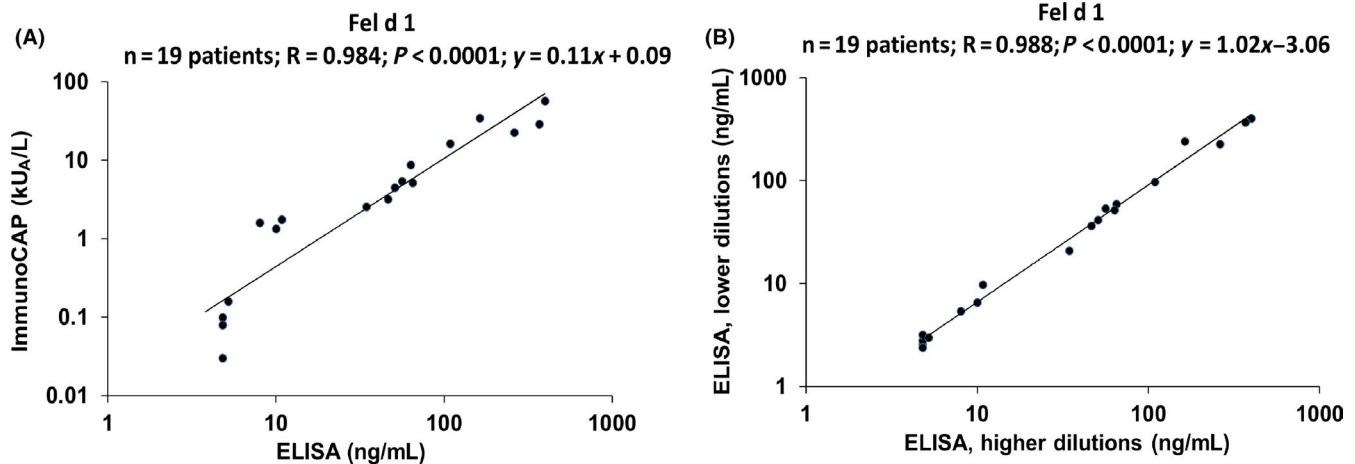


FIGURE 2 Correlation of Fel d 1-specific IgE levels determined in sera from 19 cat allergic patients by ImmunoCAP (kU_A/L) (y-axis) versus ng/ml determined by ELISA (x-axis) (A). Correlation of Fel d 1-specific IgE levels determined in lower (y-axis) and higher (x-axis) sera dilutions from 19 cat allergic patients from Table S3 (B). Correlation coefficients (r), regression equations, and statistical significances (P values) are shown in the top upper corner of graph

flexible testing of different allergen molecules which can be kept on stock and used depending on the clinical questions and diagnostic needs required. To achieve these goals, the authors will make efforts to make available the key reagents for the standard curve (ie, IgEmoAb and rBet v 1) to the community. In summary, we provide a simple, inexpensive, sensitive and accurate method for measuring allergen-specific IgE concentrations in serum and body fluids to facilitate the diagnosis of IgE-associated allergy in the world.

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CONFLICT OF INTEREST

RV has received research grants from HVD Life Sciences, Vienna, Austria and Viravaxx, Vienna, Austria, and serves as consultant for Viravaxx. MvH has received lecture fees from Thermo Fisher Scientific and consultancy fees from Biomay AG, Vienna, Austria, and Hycor Biomedical LLC, CA, US, outside of the submitted work. Other authors have no conflict of interest to declare.

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REFERENCES

1. Anto JM, Bousquet J, Akdis M, et al. Mechanisms of the Development of Allergy (MeDALL): Introducing novel concepts in allergy phenotypes. *J Allergy Clin Immunol*. 2017;139:388-399.
2. Westman M, Lupinek C, Bousquet J, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol*. 2015;135:e1-11.
3. Wickman M, Lupinek C, Andersson N, et al. Detection of IgE reactivity to a handful of allergen molecules in early childhood predicts respiratory allergy in adolescence. *EBioMedicine* 2017;26:91-99.
4. Grönlund H, Adéyoyin J, Reininger R, et al. Higher immunoglobulin E antibody levels to recombinant Fel d 1 in cat-allergic children

with asthma compared with rhinoconjunctivitis. *Clin Exp Allergy*. 2008;38:1275-1281.

5. Elisyutina O, Fedenko E, Campana R, et al. Bet v 1-specific IgE levels and PR-10 reactivity discriminate silent sensitization from phenotypes of birch allergy. *Allergy* 2019;74:2525-2528.
6. van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: Pros and cons in allergology. *J Allergy Clin Immunol*. 2017;140:974-977.
7. Laffer S, Hogbom E, Roux KH, et al. A molecular model of type I allergy: identification and characterization of a nonanaphylactic anti-human IgE antibody fragment that blocks the IgE-FcεRI interaction and reacts with receptor-bound IgE. *J Allergy Clin Immunol*. 2001;108:409-416.
8. Grönlund H, Bergman T, Sandström K, et al. Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. *J Biol Chem*. 2003;278:40144-40151.
9. Levin ME, Botha M, Basera W, et al. Environmental factors associated with allergy in urban and rural children from the South African Food Allergy (SAFFA) cohort. *J Allergy Clin Immunol*. 2019;145:415-426.
10. Gray CL, Levin ME, du Toit G. Egg sensitization, allergy and component patterns in African children with atopic dermatitis. *Pediatr Allergy Immunol*. 2016;27:709-715.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Highly sensitive ELISA-based assay for quantification of allergen-specific IgE antibody levels

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Methods

Expression, purification and characterisation of human allergen-specific monoclonal IgE (IgE_{mo}Ab), recombinant allergens

In the humanised monoclonal Bet v 1-specific IgE antibody, the variable region of a mouse IgG₁ antibody specific for the major birch pollen allergy (Bet v 1)^{S1}, was fused to the human epsilon heavy chain and the DNA construct was introduced into hybridoma cells by electroporation^{S1, S2}. Hybridoma cells were cultured in CD Hybridomamedium (ThermoScientific, Uppsala, Sweden) supplemented with GlutamaxTM (Thermo Scientific) into which IgE_{mo}Ab was secreted. A monoclonal anti-human IgE antibody, mAb12^{S2, S3}, was used to purify IgE_{mo}Ab from cell culture supernatants by single step affinity chromatography. Purified IgE_{mo}Ab was dissolved in PBS and kept frozen at -20°C until use. The concentration of purified IgE_{mo}Ab was determined by BCA assay (Pierce, Rockford, IL, USA). In order to check the purity of IgE_{mo}Ab, 1 and 2 µg purified antibody along with a protein weight ladder (PageRuler prestained Protein Ladder Plus, Fermentas, St. Leon-Rot, Germany) were loaded onto a 15% polyacrylamide gel and separated by SDS-PAGE under non-reducing and reducing conditions, followed by Coomassie Blue staining. Likewise, 10 and 20 ng IgE_{mo}Ab were loaded onto a 15% polyacrylamide gel and separated by SDS-PAGE, followed by semi-dried transfer to a nitrocellulose membrane (GE Healthcare, Chicago, IL, USA). Human IgE was detected upon incubation with 1:5000 Anti-human IgE-HRP (Seracare, Milford, MA, USA).

The far UV circular dichroism (CD) spectrum of purified IgE_{mo}Ab was determined using a Jasco J-810 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan). An aliquot of 400 µL of a solution with a concentration of 200 µg/mL IgE_{mo}Ab in 10 mM NaH₂PO₄ (pH=7) was placed in a 1mm path length quartz cuvette and its spectrum was measured from 260 to 190 nm with a

resolution of 0.5 nm at 50 nm/min. The measurement was repeated three times and averaged. Purified recombinant Bet v 1 was obtained from BIOMAY (Vienna, Austria) and rFel d 1 was expressed and purified as described^{S4}.

Demographic and clinical characterization of allergic patients and non-allergic control subjects

Experiments were conducted with serum samples of clinically well characterized cat (n = 19) and birch (n = 19) allergic patients from the Russian Federation after having obtained informed consent of the patients or patient`s parents in the case of children. Blood was collected at certified medical centers by licensed medical personnel (Institute of Immunology FMBA of Russia, Moscow; City Hospital #52, Moscow, and Ural State Medical University, Ekaterinburg, Russian Federation). The study was approved by the Ethic Committee of NRC Institute of Immunology FMBA of Russia- protocol No. 3, dated 09.03.2017, performed according to declaration of Helsinki and all participants gave their written informed consent. Each patient completed a questionnaire that contained demographic data, such as age and gender and questions about the symptoms that patients experienced in contact with birch and/or cat. Inclusion criteria were reported clinical symptoms of either asthma, rhinitis, conjunctivitis and/or dermatitis upon exposure to cat or birch. Full description of clinical and demographic characteristics of patients with birch and cat allergy, including phenotypes (asthma, rhinitis, conjunctivitis or dermatitis) are shown in Table S1. Representative sera were selected to comprise the range from low to high of allergen-specific IgE levels. Serum from a non-allergic individual was included as control.

Establishment of IgE_{moAb}-based standard curves for quantification of sIgE by ELISA

Standard curves for the quantification of allergen-specific IgE were established using ELISA plate coated with rBet v 1 and defined dilutions containing different concentrations of IgEmaAb (i.e., 2.42 µg/ml, 242 ng/ml, 121 ng/ml, 60.5 ng/ml, 24.2 ng/ml, 12.1 ng/ml, 6.05 ng/ml, 3.02 ng/ml, 2.42 ng/ml). The dilutions of IgEmaAb were prepared in 2% BSA in 0.5% PBS-Tween buffer to prevent loss of IgEmaAb due to binding to the plastic vials used for preparing the dilutions (Safe-Lock Tubes 1.5 ml Cat. № 0030120086, Eppendorf, Hamburg, Germany). In pilot experiments, different concentrations of rBet v 1 (1 µg/ml, 2 µg/ml, 5 µg/ml) were used for coating of ELISA plates (96 well F-bottom microplate, Greiner Bio-One GmbH, Germany) and a concentration of 1 µg/ml of Bet v 1 was found to be suitable for obtaining a standard curve. After coating with rBet v 1 in Bicarbonate buffer plates were blocked with 1% BSA in 0.5% PBS-Tween (200 µl/well) for 2.5 h at 37 °C. Different dilutions of IgEmaAb in TBS containing 0.5% v/v Tween 20 (TBS-T) and 2% BSA were added to the ELISA plates and bound IgE was detected with HRP-coupled goat anti-human IgE antibodies diluted 1 : 2500 (KPL, Gaithersburg, MD). The OD values corresponding to bound antibodies were measured at 405 and 490 nm on ELISA reader CLARIOstar (BMG Labtech, Germany). All determinations were conducted in triplicates, and results were expressed as mean values with a variation coefficient of less than 5%. Based on the ELISA results the standard curve was established allowing to measure and quantify allergen-specific IgE. The equation describing the Standard Curve was determined as $y = -60.302 - 50.933x - 30.103x^3 + 60,7969e^x$ using TableCurve 2D Software.

Quantification of sIgE in sera from allergic patients by ELISA

For coating the ELISA plates, 1 µg/ml of rBet v 1 or 1 µg/ml of rFel d 1 were incubated in Bicarbonate buffer, (100 µl/well) overnight at 4°C. After the plates were washed 3 times with

200µl/well of PBS-Tween unspecific bindings were blocked with 1%BSA in PBS-Tween (200µl/well) for 2.5h, on 37°C. Patient`s serum diluted in 2% BSA-PBS-Tween (dilutions as described below) and set of dilutions of IgE_{mo}Ab were added (100µl/well) and incubated at 4°C overnight. Plates were then washed 5 times with 200µl/well of PBS-Tween. For detection of human IgE antibodies anti-human IgE-KPL (coupled with horse radish peroxidase) in dilution 1:2.500 in 0.5%BSA-PBS-Tween was incubated for 1h at 37°C and then for 1h at 4°C. Plates were washed 5 times with 200µl/well of PBS-Tween followed by adding 100µl/well ABTS buffer and then were read after 5min, 10min, 15 min, 20min at OD 405 and 495 on ELISA reader CLARIOstar (BMG Labtech, Germany). Each patient`s serum was diluted 1:2 and 1:4 (if ImmunoCAP result for this sample were less than 50 kU_A/L) or 1:8 and 1:16 (if ImmunoCAP result for this sample were more than 50 kU_A/L). Using the formula $y = -60.302 - 50.933x - 30.103x^3 + 60,7969e^x$ for each OD sample obtained by ELISA we calculated the IgE concentration in ng/ml. Values with OD>2.8 after 10 min were excluded from the further calculations since curve was already reaching the plateau. Considering the two dilutions for each serum sample and exclusion of samples with OD>2.8, we obtained 34 samples for IgE reactivity testing to Bet v 1. For comparison, IgE levels to Bet v 1 for the same patients` sera were determined using quantitative IgE ImmunoCAP (Phadia 100) and by ImmunoCAP ISAC chip (Thermofisher, Uppsala, Sweden) according to manufacturer`s instructions.

A dilution series of IgE_{mo}Ab (2.42 µg/ml, 242 ng/ml, 24.2 ng/ml, 2.42 ng/ml) were also tested using ImmunoCAP technology. We noted that the ImmunoCAP determination of the concentrations of IgE_{mo}Ab were different (i.e., yielding approx. 30%) from the results obtained by microBCA protein determination which may be due to differences between the ImmunoCAP IgE standard and IgE_{mo}AB. According to the factor 1kU_A/l=7 ng/ml (i.e., real conversion

factor) obtained by measuring the IgE_{mo}Ab dilution by ImmunoCAP, the calculation for conversion of ImmunoCAP results from kU_A/L to ng/ml were made.

Using a set of sera (n=19) from clinically well-characterized cat allergic patients (supplementary Table 3) Fel d 1-specific IgE levels were quantified with the IgE ELISA as described above and compared with results obtained by quantitative IgE ImmunoCAP measurements. Considering the two dilutions for each serum sample and exclusion of samples with OD>2.8, we obtained 37 samples for IgE reactivity testing to Fel d 1. An OD of 0.05 was determined as a cut-off value for specific IgE reactivity by ELISA using serum dilutions from a non-allergic blood donor.

Measurement of allergen-specific IgE levels by ImmunoCAP and ImmunoCAP ISAC technology

In order to compare IgE levels determined by ImmunoCAP and the quantitative IgE ELISA, exactly the same recombinant allergen preparations (i.e., rBet v 1, rFel d 1) were used for the ELISA and for preparing ImmunoCAPs using Streptavidin CAPs as described⁵⁵. Two µg of biotin-labelled recombinant allergen rBet v 1 or rFel d 1 were added to the Streptavidin CAPs and incubated 30 minutes at RT. 150 µl of sera from 19 patients with cat allergy and 19 patients with birch pollen allergy and also set of dilutions of IgE_{mo}Ab were tested by ImmunoCAP Phadia-100 machine as described by the manufacturer (Thermo Fisher Scientific, Waltham, USA). Nineteen patients with birch pollen allergy were additionally tested by ImmunoCAP ISAC technology according to manufacturer's instructions (Thermo Fisher Scientific, Waltham, USA).

Statistical analysis

Correlation between ELISA results, ImmunoCAP and ImmunoCAP ISAC chip were assessed using Spearman's correlation coefficient. Correlation coefficients (r), regression equations, and statistical significances (p values) were determined (SPSS 11.5 for Windows, Spearman Correlation). A p value <0.05 was considered significant.

References:

- S1. Laffer, S. *et al.* Molecular characterization of Bip 1, a monoclonal antibody that modulates IgE binding to birch pollen allergen, Bet v 1. *J. Immunol.* **157**, 4953–62 (1996).
- S2. Laffer, S. *et al.* A molecular model of type I allergy: identification and characterization of a nonanaphylactic anti-human IgE antibody fragment that blocks the IgE-FcεRI interaction and reacts with receptor-bound IgE. *J. Allergy Clin. Immunol.* **108**, 409–16 (2001).
- S3. Lupinek, C. *et al.* Trimolecular complex formation of IgE, FcεRI, and a recombinant nonanaphylactic single-chain antibody fragment with high affinity for IgE. *J. Immunol.* **182**, 4817–4829 (2009).
- S4. Grönlund, H. *et al.* Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. *J. Biol. Chem.* **278**, 40144–51 (2003).
- S5. Erwin, E. A. *et al.* Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. *J. Allergy Clin. Immunol.* **115**, 1029–35 (2005).

Supplementary Figures and Tables

Supplementary Figure 1. Coomassie-stained SDS-PAGE of purified IgE_{mo}Ab under non-reducing and reducing conditions. M, molecular weight marker (kDa) (**A**). Nitrocellulose-blotted IgE_{mo}Ab under non-reducing and reducing conditions was detected by anti-human IgE-HRP. Molecular weights are indicated on the left margins (**B**) Analysis of IgE_{mo}Ab by circular dichroism. The mean residue molar ellipticities (γ -axis: degree cm² dmol⁻¹) were recorded for a range of wavelengths (χ -axis) (**C**).

Supplementary Figure S2. Recording of standard curves at different time points. A series of dilutions of IgE_{mo}Ab (χ -axis: ng/ml) was reacted with ELISA plate-bound rBet v 1 and IgE binding (γ -axis: optical density (OD) values corresponding to bound IgE) was measured at different time points (5, 10, 15 and 20 min).

Supplementary Table S1. Demographic and clinical characterization of birch pollen (B1-B19) and cat allergic (C1-C19) patients.

Supplementary Table S2. Bet v 1-specific IgE levels determined by ELISA, ImmunoCAP and ImmunoCAP ISAC for birch pollen allergic patients

Supplementary Table S3. Fel d 1-specific IgE levels determined by ELISA, ImmunoCAP and ImmunoCAP ISAC for cat allergic patients

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179 **Supplementary Table S4.** Fel d 1-specific IgE levels determined by ELISA shown as optical
180 density (OD) values for cat allergic patient's sera in dilutions 1:2 and 1:4 or 1:8 and 1:16 for C3.

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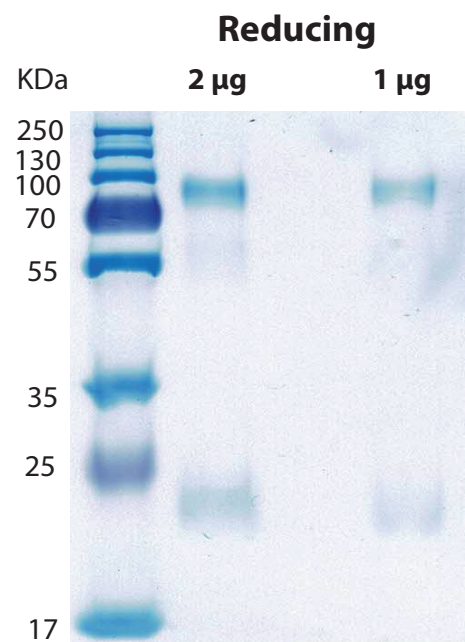
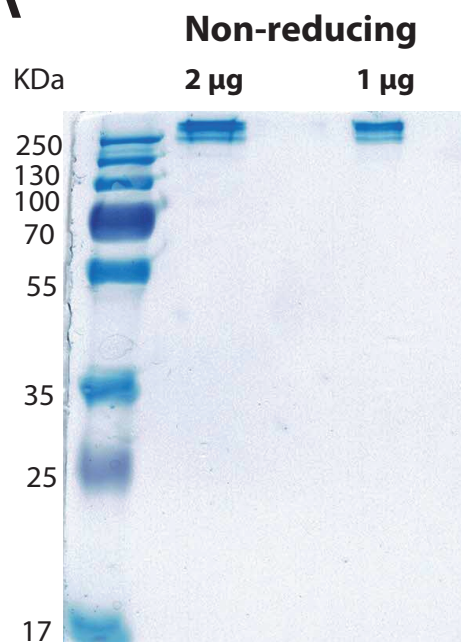
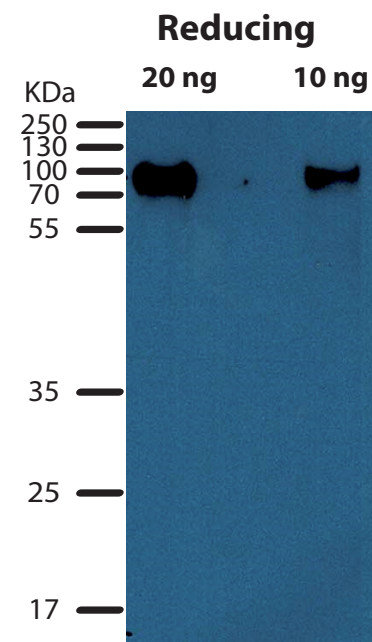
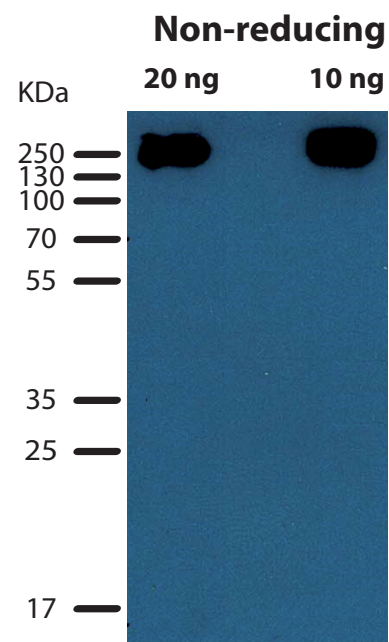
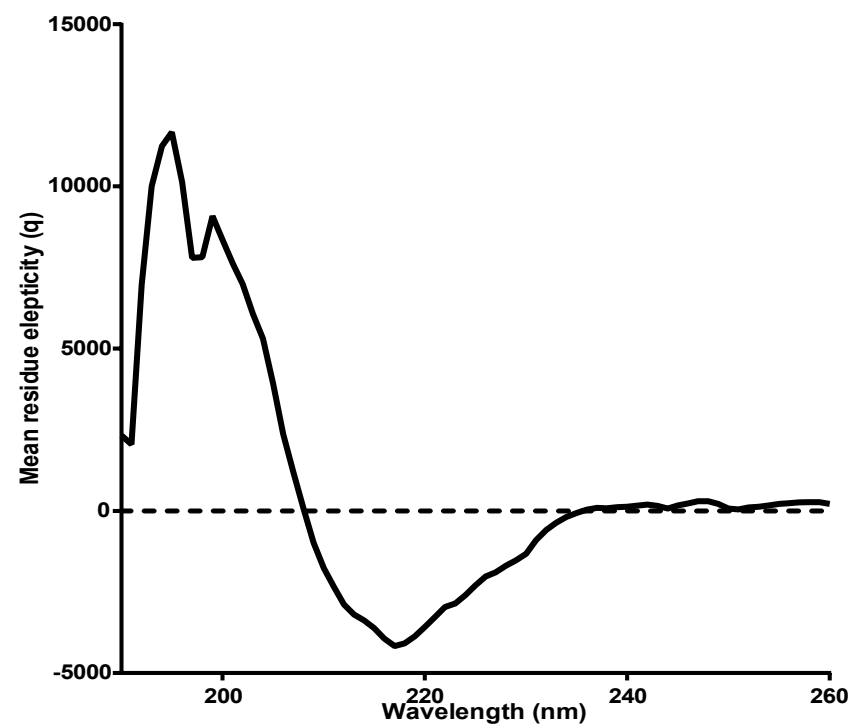
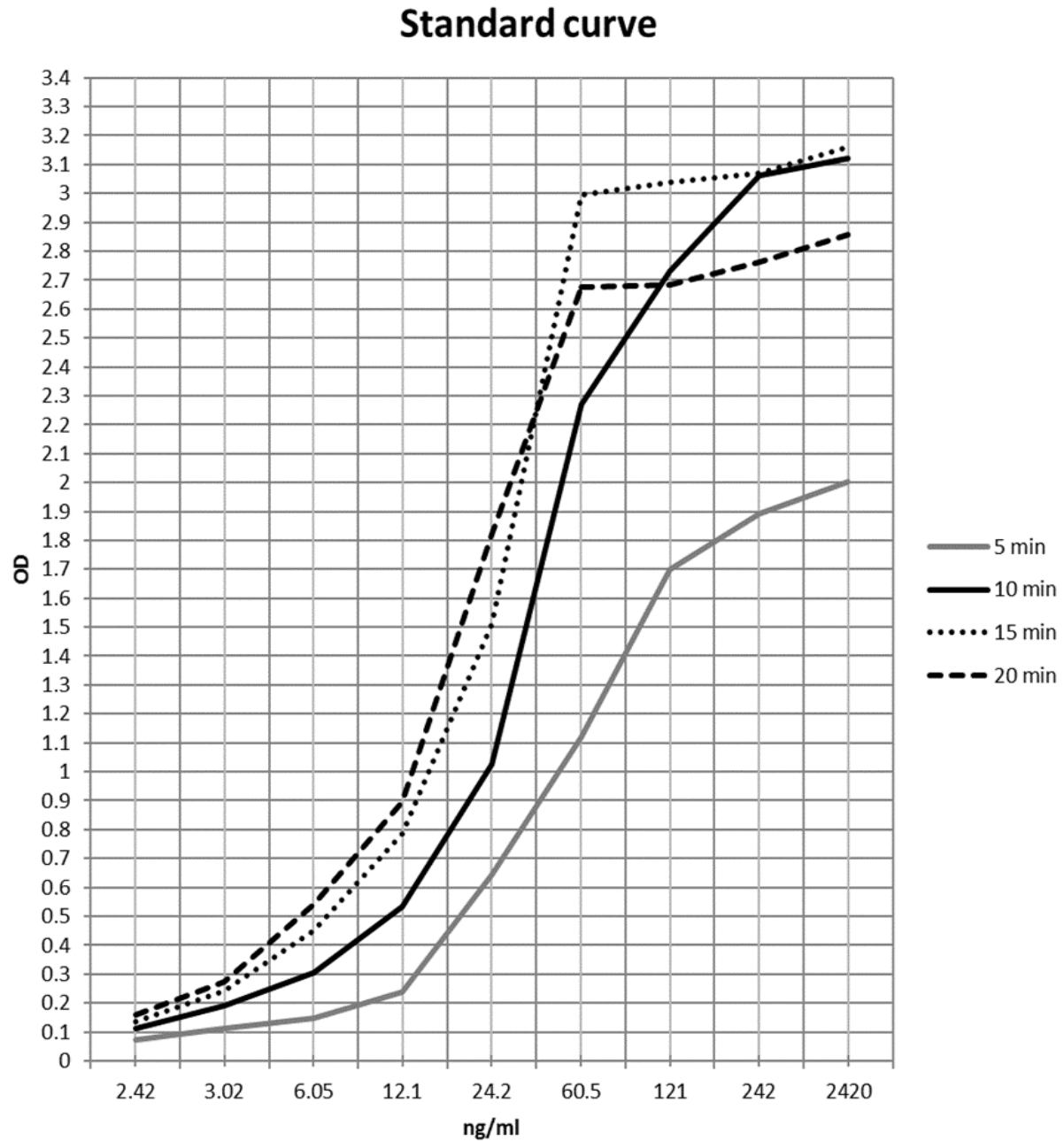
A**B****C**

Figure S1



Supplementary Figure S2. Recording of standard curves at different time points. A series of dilutions of IgEmoAb (x-axis: ng/ml) was reacted with ELISA plate-bound rBet v 1 and IgE binding (y-axis: optical density (OD) values corresponding to bound IgE) was measured at different time points (5, 10, 15 and 20 min).

8 **Supplementary Table S1.** Demographic and clinical characterization of birch pollen (B1-B19)
9 and cat allergic (C1-C19) patients.

	Sex	Age	Allergy clinical symptoms					Other allergies			
			Asthma	Rhinitis	Conj.	Dermat.	Dust	Pet	Pollen	Food	Mold
Patients with Birch Pollen Allergy											
B1	m	10	neg	pos	pos	neg	neg	pos	pos	pos	pos
B2	m	10	pos	pos	pos	pos	neg	pos	pos	neg	neg
B3	m	14	neg	pos	pos	neg	pos	pos	pos	pos	pos
B4	m	15	pos	pos	pos	pos	pos	pos	pos	neg	pos
B5	f	16	neg	pos	pos	neg	neg	pos	pos	pos	neg
B6	m	12	neg	pos	pos	neg	neg	pos	pos	pos	neg
B7	f	15	neg	pos	pos	neg	pos	pos	pos	pos	neg
B8	f	12	neg	pos	pos	pos	neg	pos	pos	pos	neg
B9	m	10	neg	pos	pos	neg	neg	pos	pos	neg	neg
B10	m	11	pos	pos	pos	neg	neg	pos	pos	pos	pos
B11	m	15	neg	pos	pos	neg	pos	pos	pos	neg	neg
B12	f	11	pos	pos	pos	pos	neg	pos	pos	pos	neg
B13	m	15	pos	pos	pos	neg	pos	pos	pos	neg	neg
B14	f	10	pos	pos	pos	pos	pos	pos	pos	pos	neg
B15	f	11	neg	pos	pos	neg	neg	pos	pos	neg	neg
B16	f	11	neg	pos	pos	neg	neg	pos	pos	neg	neg
B17	f	11	pos	pos	pos	neg	neg	pos	pos	neg	neg
B18	m	10	neg	pos	pos	pos	neg	pos	pos	neg	neg
B19	f	15	neg	pos	pos	pos	pos	pos	pos	pos	neg
Patients with Cat Allergy											
C1	m	29	pos	neg	pos	neg	pos	pos	pos	pos	pos
C2	m	26	pos	pos	pos	pos	pos	pos	pos	n/d	n/d
C3	f	25	pos	pos	pos	pos	pos	pos	pos	n/d	pos
C4	f	62	pos	pos	pos	pos	n/d	n/d	n/d	n/d	n/d
C5	f	35	pos	pos	pos	pos	pos	pos	pos	n/d	n/d
C6	f	27	pos	pos	pos	pos	pos	pos	pos	n/d	n/d
C7	m	21	pos	pos	pos	neg	pos	pos	pos	n/d	n/d
C8	m	29	neg	pos	pos	pos	pos	pos	neg	n/d	n/d
C9	m	24	pos	pos	pos	pos	pos	pos	pos	pos	n/d
C10	f	57	neg	pos	pos	neg	pos	pos	pos	n/d	n/d
C11	m	47	neg	pos	pos	neg	neg	neg	pos	n/d	n/d
C12	m	20	pos	pos	pos	neg	pos	pos	pos	n/d	n/d
C13	m	31	pos	pos	neg	neg	neg	pos	n/d	n/d	neg
C14	m	20	pos	pos	pos	neg	pos	pos	pos	pos	n/d
C15	m	22	pos	pos	neg	neg	pos	pos	neg	n/d	n/d
C16	m	20	pos	pos	neg	neg	pos	pos	pos	pos	n/d
C17	f	38	neg	pos	pos	neg	pos	pos	pos	n/d	n/d
C18	f	40	pos	pos	pos	neg	n/d	n/d	n/d	n/d	n/d
C19	m	18	pos	pos	pos	neg	pos	pos	pos	n/d	n/d

Supplementary Table S2. Bet v 1-specific IgE levels determined by ELISA, ImmunoCAP and ImmunoCAP ISAC for birch pollen allergic patients (B1-B19)

Patient ID	OD Mean	ng/ml	ImmunoCAP kU _A /L	ISAC ISU
B1	2.209	902.4	346	139.9
B2	0.867	329.6	64	11.1
B3	0.828	313.6	62.3	52.5
B4	0.594	212.8	60.5	57.2
B5	0.859	326.4	59.7	59.9
B6	1.014	97.2	48	45.8
B7	0.88	334.4	75.7	78.2
B8	2.595	1536	331	153.5
B9	1.035	396.8	16.1	20.3
B10	1.576	142.4	46	22.7
B11	0.59	52.4	9.69	16.3
B12	1.058	101.2	42.1	32.8
B13	0.268	20	3.07	3.7
B14	0.943	90	33	11.4
B15	2.742	1966.4	430	148.1
B16	0.306	23.2	2.64	4.1
B17	0.199	14	1.68	3.3
B18	1.238	467.2	146	52.7
B19	2.703	1838.4	342	144.3

Supplementary Table S3. Fel d 1-specific IgE levels determined by ELISA and ImmunoCAP for cat allergic patients (C1-C19) and non-allergic donor (D1)

Patient ID	OD Mean	ng/ml	ImmunoCAP kU _A /L
C1	0.703	64.8	5.20
C2	1.816	162.8	34.50
C3	1.041	398.4	57.20
C4	0.059	4.8	0.03
C5	0.059	4.8	0.08
C6	0.687	63.2	8.79
C7	0.621	56	5.43
C8	0.153	10.8	1.76
C9	1.143	108.8	16.30
C10	0.418	34.4	2.55
C11	0.064	4.8	0.10
C12	0.061	4.8	0.08
C13	0.534	46.4	3.19
C14	0.115	8	1.60
C15	0.069	5.2	0.16
C16	0.145	10	1.34
C17	0.573	50.8	4.50
C18	2.331	260.8	22.80
C19	0.703	368	29.00

Donor ID		
D1	0.043	0.001

Supplementary Table S4. Fel d 1-specific IgE levels determined by ELISA shown as optical density (OD) values for cat allergic patient's sera in dilutions 1:2 and 1:4 or 1:8 and 1:16 for C3.

Patient ID	Serum Dilution	OD Mean	ng/ml
C1	1:2	1.262	59.4
C1	1:4	0.703	64.8
C2	1:2	2.734	242.6
C2	1:4	1.816	162.8
C3	1:8	2.100	403.2
C3	1:16	1.041	398.4
C4	1:2	0.067	2.6
C4	1:4	0.059	4.8
C5	1:2	0.075	2.8
C5	1:4	0.059	4.8
C6	1:2	1.090	52
C6	1:4	0.687	63.2
C7	1:2	1.131	54
C7	1:4	0.621	56
C8	1:2	0.264	9.8
C8	1:4	0.153	10.8
C9	1:2	2.057	97
C9	1:4	1.143	108.8
C10	1:2	0.491	21
C10	1:4	0.418	34.4
C11	1:2	0.091	3.2
C11	1:4	0.064	4.8
C12	1:2	0.062	2.4
C12	1:4	0.061	4.8
C13	1:2	0.779	36.6
C13	1:4	0.534	46.4
C14	1:2	0.156	5.4
C14	1:4	0.115	8.00
C15	1:2	0.082	3.00
C15	1:4	0.069	5.20
C16	1:2	0.189	6.6
C16	1:4	0.145	10.00
C17	1:2	0.879	41.8
C17	1:4	0.573	50.8
C18	1:2	2.695	226.6
C18	1:4	2.3331	260.8
C19	1:2	2.979	375.8
C19	1:4	2.569	368
Donors ID			
D1	1:2	0.045	
D1	1:4	0.043	

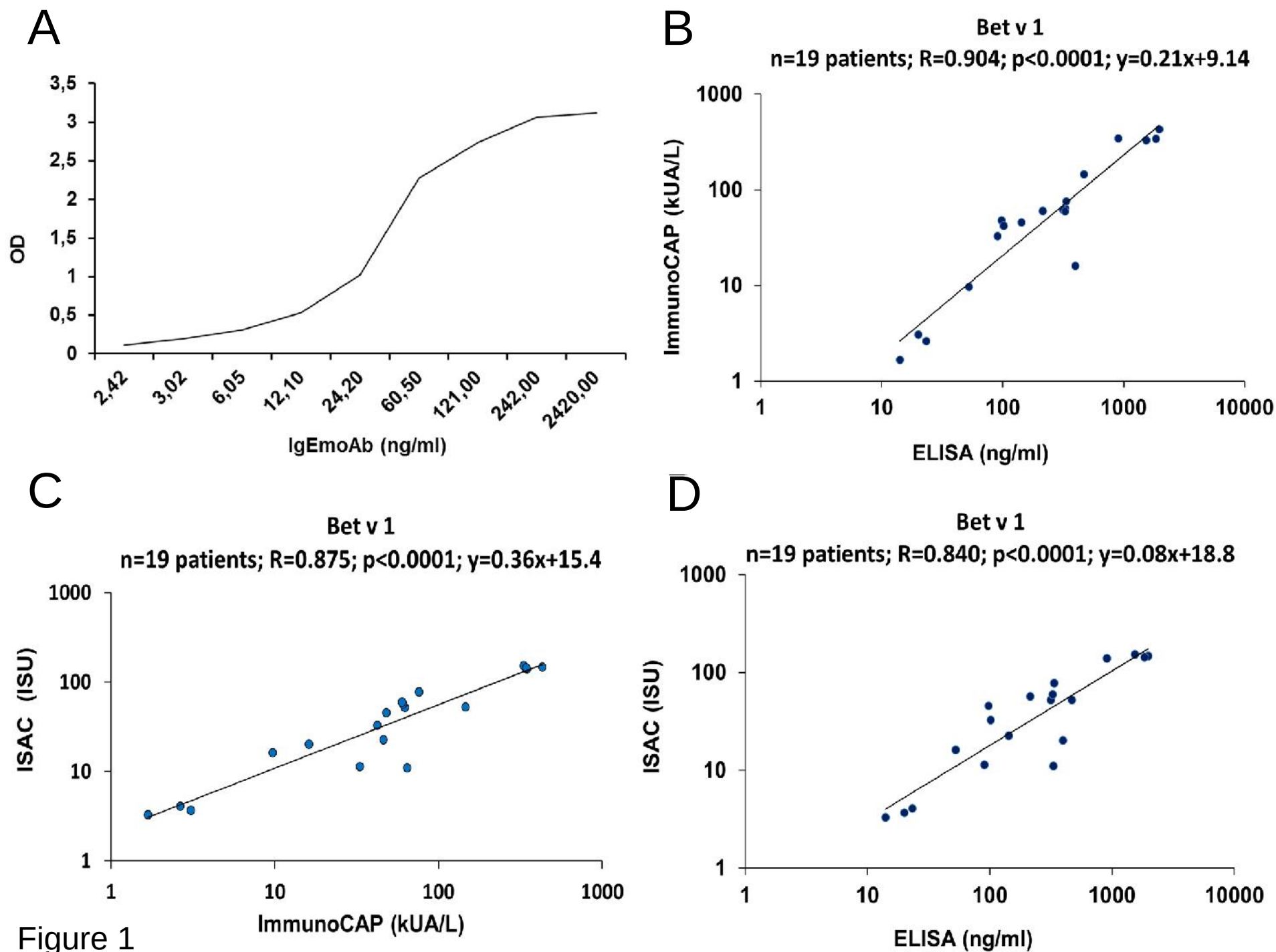


Figure 1

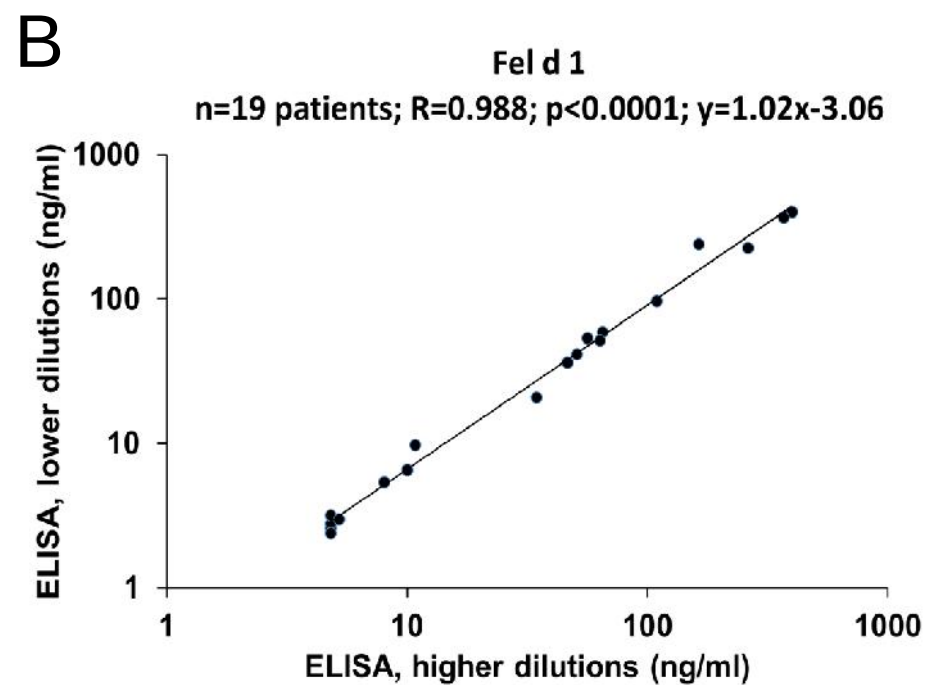
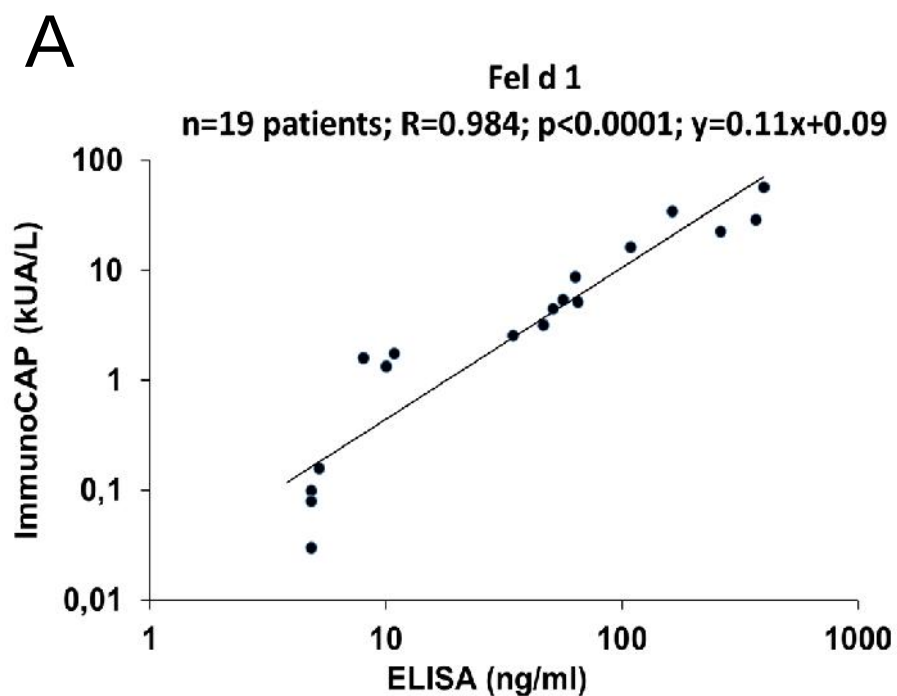


Figure 2