Biosensors and Bioelectronics 166 (2020) 112438

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

Highly sensitive optoelectrical biosensor for multiplex allergy diagnosis

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ARTICLE INFO

Keywords:
In vitro biosensing
Allergens
IgE
IgG
Multiplex
Component-based diagnostics

ABSTRACT

Compact multiplexed biosensors systems hold great potential for diagnosis of diseases where the detection of multiple biomarkers is required. Hypersensitivity Immunoglobulin E mediated syndromes are primary immunodeficiency disorders associated with sensitization to allergens. Assessing immunoglobulin E (IgE) sensitization to allergens is an important strategy for allergy diagnosis. Here, we report for the first time a reliable, flexible and cost-effective optoelectrical biosensor system for the simultaneous determination of total and allergen-specific IgE and IgG, antibodies using an immunogold-silver signal amplification method. The biosensor was constructed on a regular digital versatile disc (DVD) to immobilize a panel of 12 allergen extracts or pure proteins in microarray format, as a proof of concept. The multiplexed biosensor showed a limit of detection of 0.26 IU/mL (624 pg/mL) and 14 ng/mL for IgE and IgG antibodies, respectively. The system was successfully applied in a cohort of 127 human serum samples, showing good sensitivity (97.6%) as well as specificity (85.7%), and an excellent area under the curve (AUC) value was found at 0.977 (confidence interval, CI 0.957 to 0.990) as compared and validated with a reference clinical immunofluorescence assay, confirming an excellent correlation between both techniques. The multiplex biosensor system with on-demand panel composition can be used fully autonomously in clinical or mobile laboratory settings without the need for any additional medical equipment, with which could make it suitable for massive allergy screening campaigns to better define sensitization profiles.

1. Introduction

Allergy covers a wide spectrum of diseases, such as allergic rhinitis, allergic asthma, atopic dermatitis, urticaria and contact allergies. The term allergy is often used for IgE-mediated type-I hypersensitivity reactions which is the most common chronic disease in Europe. Today, more than 150 million Europeans suffer from allergy (indoor, outdoor, and/or food allergy) and the current prediction is that by 2025 half of the entire EU population will be affected and half of whom will be underdiagnosed (www.eaaci.org). This has a major impact on learning and performance in academia, leading to opportunity costs for society. In addition, the increasing prevalence of allergy also has important economic concerns due to absenteeism and presenteeism.

In vitro testing is the preferable method to ease the burden of allergy, specifically when high risk of anaphylaxis and skin is not in good conditions for testing; thereby reducing the number of undiagnosed people and further decrease medical treatment costs. The estimated annual cost of the appropriate therapy for allergic diseases is on average of €125 per patient, equaling only 5% of the costs of untreated disease, allowing potential savings of up to €142 billion (Zuberbier et al., 2014).

The *in vitro* diagnosis of IgE-mediated allergic diseases is useful in the identification of the causative allergen. In fact, it is the only method able to determine directly the levels of allergen-specific IgE (sensitization), providing better IgE profile of the patient what is in line with the

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https://doi.org/10.1016/j.bios.2020.112438
Received 14 May 2020; Received in revised form 3 July 2020; Accepted 7 July 2020
Available online 15 July 2020
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precision Medicine statements. In fact, a positive sIgE response accompanied with a history of allergic symptoms make the diagnosis of allergy clinically relevant (Ansotegui et al., 2020).

The *in vitro* tests are designed to either detect single sensitization (single-plex) or identify simultaneously a pre-defined panel of allergens (multiple sensitization). Most of the *in vitro* assays used in the clinical practice are based on single-plex configuration to detect allergen specific IgE one by one, using a labeled anti-human IgE antibody leading to either colorimetric, fluorescent or chemiluminiscent detection modes. In this context, Immuno-CAP (Thermo Fisher Scientific) now is perceived as the "gold standard method" for single-plex *in vitro* IgE testing. Despite their methodological differences, other fully automated systems such as IMMULITE 2000/2500 (Siemens) show also significant correlation with respect to detect sIgE of inhalant and food allergens in qualitative comparison studies (Park et al., 2018). However, the weaknesses of both systems are their single-plex and semiquantitative character, low linear range, cost per assay-allergen, the large amount of sample required to perform sensitization profiles especially in pediatric studies, and assay running time that can takes from 65 to 100 min for a single sample. The functional sensitivity of both systems is close to 0.2 IU/mL and the tests are always carried out in fully equipped laboratory settings. The price for instrumentation ranges from 15,000 € to more than 100,000 €, depending on the performance and working capacity. The reversed enzyme allergosorbent test with liquid-phase allergens (Dr. Fooke-Achterrath Laboratorien GmbH) is also a reliable single-plex immunoassay using microwells, based on a sandwich ELISA. Determination of specific IgE against 500 allergen extracts and purified native and recombinant allergen components, in serum or plasma is possible with high sensitivity and specificity. The assay takes 3 h for the manual procedures (Popescu and Vieru, 2018).

Whereas single-plex systems are available from several companies, there are currently only few manufacturers that offer multiplex assays for allergy diagnostics. Multiplex approaches are interesting systems for allergy diagnosis mainly because people is rarely allergic to only one allergen (Baatenburg de Jong et al., 2011). Today, ImmunoCAP ISAC is one of the most complete multiplex platform currently available for simultaneously determination of 112 specific IgE in serum. The assay is semiquantitative, few samples are analysed simultaneously and the results are measured with a fluorescence scanner. The test has been widely used for different studies (Hemmer et al., 2018; Yamamoto-Hanada et al., 2020). However, according to the manufacturer, sIgE concentrations below 1.0 IU/mL are not reliably detectable (Jakob et al., 2015). Therefore, the over-all assay sensitivity is lower than that of the ImmunoCAP (single-plex). Other example of a multiplexed system is the MeDALL allergen-chip. The chip detects specific IgG and IgE towards 170 allergen molecules in human sera. It has technical advances in terms of specificity, sensitivity, giving results very close to *in vivo* reactivity (Lupinek et al., 2014; Siroux et al., 2017). In recent studies, allergic sensitization to inhalant allergens or components was significantly higher using the MeDALL-chip compared to ImmunoCAP results (Skrindo et al., 2015). At present, the MeDALL-chip is available only as a research tool and costs for clinical use cannot be calculated. ALEX is another *in vitro* multiplexed test designed for the diagnosis of type I allergies (Francesca et al., 2019; Heffler et al., 2018). The macroarray test includes an allergen panel of more than 295 allergen extracts and molecular allergens. The quantification is based on a colorimetric image acquisition, using CMOS sensors and expensive instrumentation. The assay time takes 3.5 h with manual processing, reaching a measuring range between 0.3 and 50 IU/mL.

As the clinical and analytical performances, costs and time required for a reliable allergy diagnosis are important issues in medical practice, highly sensitive, flexible and cost-effective biosensing systems are of great scientific interest. Indeed, the use of biosensors is gaining competitive advantages for *in vitro* allergy diagnosis according to specific IgE levels in serum (Morais et al., 2020). Besides, the concept of envisioning biosensor systems derived from consumer electronics that fulfil the requirements for the clinical practice has been explored during the last decade (Hwu and Boisen, 2018; Morais et al., 2016). In this context, to our knowledge, this is the first approach based on compact optoelectronical biosensor as versatile consumer electronics device in combination with immunogold-silver signal amplification for highly sensitive *in vitro* allergy diagnosis. Allergen microarrays are built on a regular digital compact disc *a la carte* to avoid unnecessary testing and the results read with a hacked DVD reader. As proof of concept, the precise and sensitive simultaneous determination of total and specific IgE and IgG antibodies against 12 allergens is presented. The reliability of the system was evaluated in a cohort of 127 human serum samples, this being excellent in the light of the clinical records and the results of the reference *in vitro* test.

2. Materials and methods

2.1. Allergens and immunoreagents

The multiplexed optoelectrical sensor comprised of allergen extracts and pure proteins from a wide range of environmental inhalant allergens immobilized on a digital versatile disc. The extracts and pure proteins were obtained from ALK-Abelló (Hørsholm, Denmark). Gold spherical nanoparticles (5 nm), bovine serum albumin (BSA), trizma base, Tween-20, and silver enhancer reagents were obtained from Sigma-Aldrich (Madrid, Spain). Four monoclonal anti-human IgE antibodies were used; mAb1 was from Dr. Fooke Laboratorien GmbH (Neuss, Germany), mAb2 and mAb3 from Abcam (Cambridge, United Kingdom), and mAb4 from Eurofins Ingenasa, S.A. (Madrid, Spain). The monoclonal antibody anti-human IgG was also from Abcam.

WHO reference IgE standard 11/234 from the National Institute for Biological Standards and Control (Hertfordshire, United Kingdom) is intended to standardise assays for serum IgE expressed in IU/mL. The IgG standard was obtained by affinity chromatography purification of human sera (Sigma-Aldrich, Madrid, Spain), using HiTrap Protein G HP columns (GE Heathcare, Chicago, Illinois, USA). The purified IgG standard was calibrated against the international reference preparation "1st WHO IRP 67/86 for human IgG expressed in μg/mL. The conjugation of the detector antibodies (monoclonal anti-human IgE and IgG) to 5 nm gold particles was performed as previously described (Dobosz et al., 2015).

Printing buffer was 100 mM sodium carbonate/bicarbonate buffer, pH 9.6, assay and washing buffer (PBST) was 10 mM sodium phosphate buffer, 150 mM NaCl, 0.05%, Tween-20, pH 7.5.

2.2. Calibration

The calibration curves for total IgE and IgG were built, performing the corresponding sandwich immunoassays. For that, allergen-specific IgE levels expressed as IU/mL were determined, using the WHO standard as calibrator for total IgE determination, involving heterologous interpolation as a calibration method. The allergen-specific IgG levels expressed as μg/mL were determined, using IgG standard for total IgG determination, involving also a heterologous interpolation. The limit of detection (LOD) and quantification, (LOQ) was calculated measuring 10 blanks and interpolating the mean of the signal plus 3 and 10 times the standard deviation of the blanks to the calibration curve, respectively.

2.3. Human serum samples

Blood samples from 127 adults were collected in red-top tubes (BD Diagnostics, Madrid, Spain), incubated at room temperature for 60 min to induce clotting. After centrifugation at 2000 rpm for 15 min, the serum was aliquoted into cryovials and stored at 80 °C till use. A cohort of 119 allergic patients (positive to at least one allergen) and eight controls were selected. All the subjects were previously examined by prick test or other *in vivo* test. Clinical records were also considered. This

study was approved by the Hospital Universitari i Politènic La Fe ethical review group. All experiments were performed in accordance with the relevant guidelines and regulations.

2.4. Assay protocol

The assays consist of the detection of specific allergen-IgE or IgG antibodies, using human anti-IgE or anti-IgG antibody-functionalized nanoparticles, respectively. A scheme of the assay protocol and the printing layout is depicted in Fig. 1. First, 40 nL of the allergen proteins or extracts previously diluted 1/10 in printing buffer were dispensed onto the polycarbonate surface of standard DVDs (CD Rohling-up GmbH, Saarbrücken, Germany), in microarray format (14 arrays per disk of 6 × 7 spots), using a noncontact printing device (AD 1500 Bio-Dot, Inc., Irvine, CA). The spots were 500 μm in diameter with a center-to-center distance of 1.0 mm, achieving an array density of 4.0 spot/mm^2. Within each microarray, spots for the determination of specific IgE and IgG for 12 allergens (three replicates each), total IgE and IgG and a negative control were included. After printing, the disks were incubated for 16 h at 4 °C. Before running the assay, the disk was thoroughly washed with PBST, rinsed with deionized water, and dried by centrifugation at 800 rpm.

The assay starts adding on the array 25 μL of the serum sample. The sample was previously conditioned with Tween-20 to a final concentration of 0.125% (v/v) or diluted 1/50 (v/v) in PBS-T for the detection of specific IgE and IgG antibodies, respectively. After 45 min, the disk was washed with PBST and deionized water. Allergen-specific IgE and IgG were detected by adding 25 μL of gold labeled anti-human IgE (Au-anti-hIgE) and gold labeled anti-human IgG (Au-anti-hIgG), respectively. After 15 min, the disk was washed as previously. The immunoreaction was developed by the immunogold-silver staining method forming silver precipitates. After 6 min, the reaction was stopped by washing the disk thoroughly with water.

2.5. Sensing concept and data analysis

The optoelectrical biosensor consists of three main parts, that are (1) a high-speed data acquisition card (DAQ); (2) a power and interface card (PIC); and (3) an optical disc drive (ODD). Fig. 2 shows a scheme of the optoelectrical biosensor.

The DAQ comprises three main functional blocks to accomplish signal detection, analog acquisition, and de-serialization and data transmission. The signal detection involves the analog processing (conditioning and pre-amplification) of the optical reflection signal obtained from the quadrant photodiode of the pickup unit of the ODD, as well as the digital detection of the trigger signal for each sector/microarray on the disc. The analog acquisition is performed with the LT2323 Analog to Digital Converter (ADC) that digitizes the reflection signal. The de-serialization block converts the serial digital output of the SPI (Serial Peripheral Interface) from the ADC into 16-bit parallel data format. For this, a Cyclone III FPGA (Field Programmable Gate Array) is used, which also manages the configuration of the sampling rate, and the interface with the USB transceiver for high-speed data transfer.

The PIB supplies the voltage for the DAQ, the optical disc drive and the electronic components of the HUB and SATA Bridge. In addition, it provides the communication interface to the external computer via a USB Type B connector. The computer connects to the internal HUB with four USB ports. One port is plugged to the data acquisition card, and other is connected to the SATA Bridge to communicate with the optical disc drive. The other two USB ports are free to use. On the other hand, the ODD is a commercial ASUS BC-12D2HT disc drive, holds the disc, and performs the readouts. Analog signals from the ODD were extracted from identified RF test points of the quadrant photodiode of the optical pickup unit. The electrical connections were made to the DAQ with a rework in the main board of the ODD to obtain the RF differential signal.

The DVD is inserted into the ODD and a simulation of the disc recording is carried out, in the same way, as it would be done with any blank recordable disc (do Nascimento et al., 2017). The scanning of the disc is carried out by moving the laser optical pickup assembly from the inner radius to the outer, rotating the disc at a constant angular velocity. The biosensor system achieve an electronic optical resolution of 5.5 μm. The reader is controlled by custom software (BIODISK), running on a personal computer and connected to it through a USB2.0 universal serial bus interface. During this process, the laser beam ($\lambda = 650$ nm) scans the entire surface of the disc, being able to correlate the variations in optical power of the reflected beam with the concentration of specific IgE and IgG, which is proportional to the optical density of the silver precipitate. Subsequently, the reflected signal is obtained from the quadrant photodiode of the ODD's optical pick-up unit (RF differential signal) and digitized using the DAQ. The custom software works under Windows (Windows W7 and W10) and is written in Microsoft Visual C ++; it generates an image for each microarray in tiff format. Each image corresponds to one of the 14 arrays of the disk. An image/data analysis algorithm divides the image into a set of objects that identify each one inside a position of the array. The data analysis generates the digital signals and quality parameters (concentration, error calculation and quality controls) for each spot by subtracting local background from the median signal value of the spot.

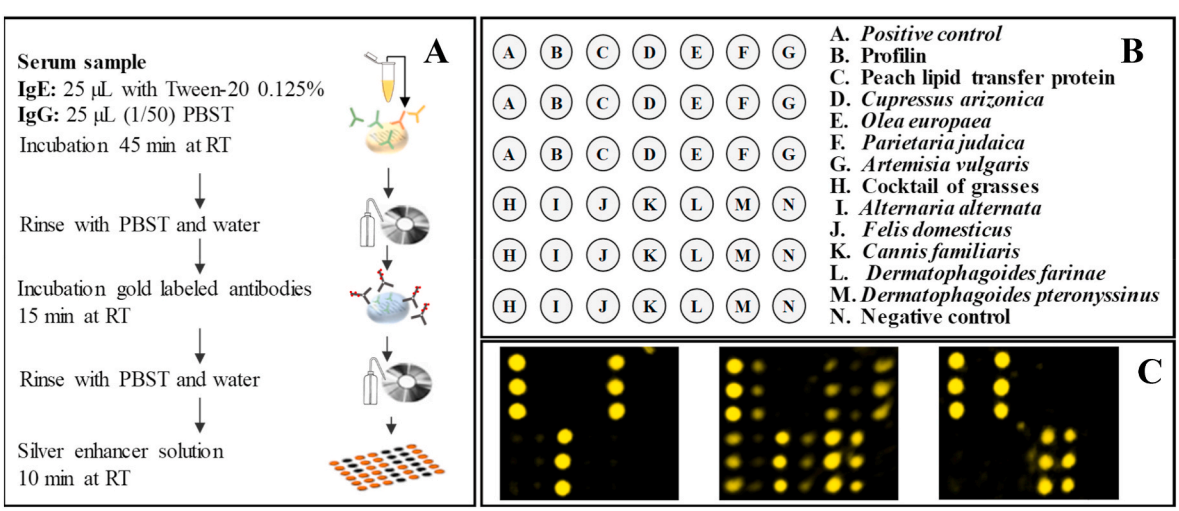

Fig. 1. A) Scheme of the assay protocol for the determination of total and specific immunoglobulin E and G. B) Layout of the allergen microarray. C) Representative images of processed microarrays after incubation with serum samples.

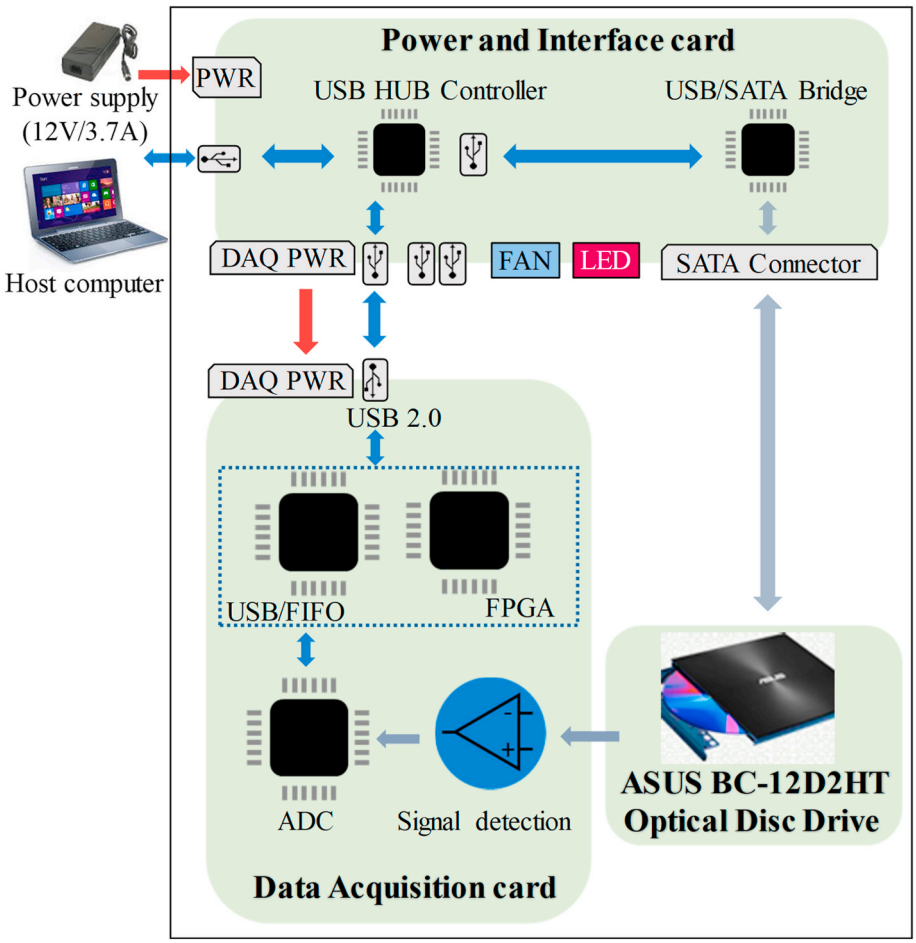

Fig. 2. Schematic of the optoelectrical biosensor.

3. Results and discussion

3.1. Calibration method

A reliable quantitative assay must report the results in units traceable to an international standard. Nowadays, it is not possible to use individual specific IgE and IgG standards for each allergen to perform the homologous calibration method. Therefore, the calibration method mostly accepted is a heterologous interpolation of specific immunoglobulins E or G from a single total immunoglobulin reference curve. In this particular case, the quantification of total IgE requires a matching pair of antibodies acting one as capture and the other as detector to develop the sandwich assay. The pair of antibodies should be compatible between them in terms of the epitopes that are recognized by each one. For this study, sixteen pairwise combinations of commercially available human anti-IgE antibodies were tested in sandwich immunoassays on DVD. Calibration curves were obtained for each pair by plotting the signal intensity against the concentration of the WHO standards. The signals were fitted to a four-parameter logistic curve (Fig. 3). The sensitivity and the linear dynamic range were the criteria for the selection of the matching pair antibodies. Fig. 3A shows the calibration curves of the assays with mAb4 as detector. The rest pairwise combinations using mAb1, mAb2 or mAb3 provided less sensitive assays (data not shown). As is observed for the sandwich assays using both the mAb2 and mAb3, the calibration curves were characterized by the short dynamic range and low sensitivity (> 70 IU/mL), measured as EC_{50}. Regarding the sensitivity, the assays using mAb1 and mAb4 showed an EC_{50} value of 6.9 and 8.2 IU/mL, respectively. The assay developed with the pair mAb1/mAb4 (capture/detector), showed the widest linear dynamic response, ranging from 0.7 to 100 IU/mL ($r^2 = 0.99$), a limit of detection of 0.26 IU/mL. The standard deviations obtained for the calibration curve were between 9% and 15% within the 1.75–100 IU/mL, and between 16% and 21% for 0.7 IU/mL and below. These performances allow the quantification of specific IgE by heterologous interpolation and thus grouping patients into classes according to the RAST classification.

Regarding total IgG quantification, a gold-labeled monoclonal antibody was also used as detector for the sandwich immunoassay. As it can be observed in Fig. 3B, the signals fit well to a four-parameter logistic curve, achieving a limit of detection of 0.014 μg/mL and dynamic response ranging from 0.025 to 25 μg/mL, following a point-to-point calculation method approach. The EC_{50} value was 0.51 μg/mL and the relative standard deviation values along the whole calibration curve were below 16%.

3.2. Determination of the analytical performances

The allergens were chosen considering the frequencies of allergen reactions in Spain, which mainly corresponds to the frequencies in South and Central Europe. The allergens belonged to different groups, including pollens and outdoor allergens, as-proof-of concept, for the evaluation of the optoelectrical biosensor.

The linearity of dilution test was performed using two pooled serum samples (n = 8) with known specific IgE concentration determined by the ImmunoCAP system. One sample contained 84.5 and 23.1 IU/mL specific IgE for *D. farinae* (*Der f*) and *D. pteronyssinus* (*Der p*), respectively, and the second 43.1 IU/mL specific LTP from peach (LTP). This study was carried out, using 4-fold serial dilutions (1/4–1/256).

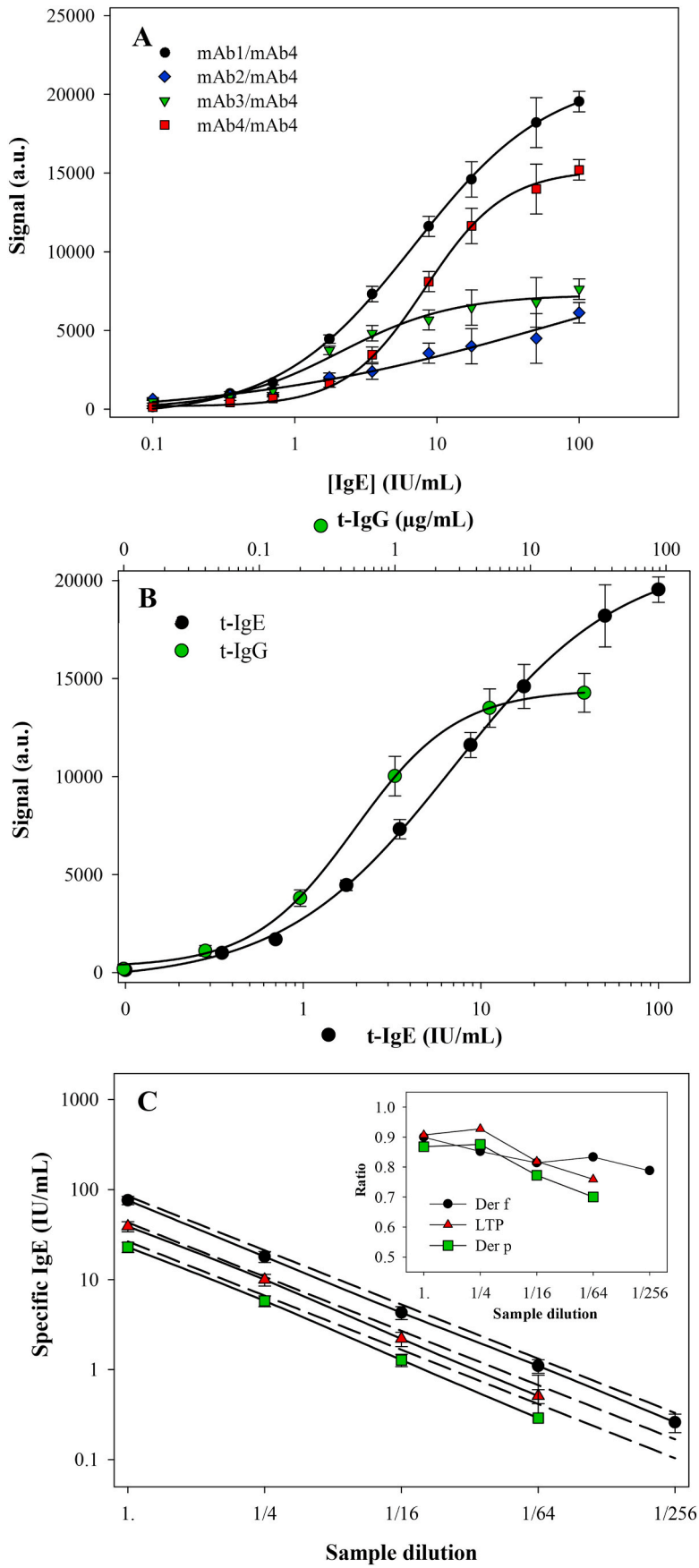

Fig. 3. A) Matching pair antibodies calibration curves with mAb4 as detector. B) Total IgE and IgG calibration curves. C) Linearity of dilution study for two pooled serum samples.

Dilutions were made in a control serum sample diluent. Measured concentrations were assessed relative to the assay standard curve. Fig. 3C shows the results of the linearity of dilution assay for the two tested samples. The solid and dashed lines represent the corresponding linearity of dilution plots for the experimental and the estimated concentrations, respectively. The experimental concentration of specific IgE was calculated, using the standard calibration curve and the estimated concentration measured by the known dilution factors. As is shown in the figure the linearity was good over a wide range of dilution, revealing that the methodology provided flexibility to test serum samples with different levels of specific IgE. The experimental/estimated concentration ratios for the different dilutions are illustrated in the inset of the Fig. 3C. As it can be seen, the experimentally determined allergen specific IgE concentrations differed slightly from the estimated concentrations, ranging from 70 to 91%. The higher ratio was obtained, as expected for the highest specific IgE concentrations (ca. 90% for > 20 IU/mL). The relative standard deviation values were below 20% for all dilutions.

The limit of detection (LOD), defined as the lowest specific IgE concentration that can be reliably estimated was determined for the 12 allergens. The LOD was between 0.26 and 0.33, being these figures slightly below the current internationally accepted cut-off concentration for allergy diagnostics (0.35 IU/ml).

3.3. Comparative evaluation studies with the reference methodology

For evaluation of our method, the cohort of 127 serum samples, 119 atopic patients with a known reactivity to one or more allergens and 8 non-atopic healthy donors, were analysed with both the multiplex optoelectrical sensor and the ImmunoCAP reference method. The correlation study was evaluated, using a statistical analysis performed with the MedCalc Software (MedCalc Ltd. Ostend, Belgium).

For this study, two comparisons with the results obtained by ImmunoCAP method were made. Fig. 4 shows the results. First, a comparison of the total IgE concentration represented in a suitable number in the cohort of patients tested, and second, a comparison of the capacity to quantify the concentration of specific IgE for the panel of allergens. Regarding total IgE concentration, both methods are comparable ($r = 0.99$; $P < 0.001$), reaching a good significance limited to 1000 IU/mL (Fig. 4A), concentration from which the system reaches recovery values around 60%. Nevertheless, most of the total IgE values were found between 10 and 250 IU/mL (mean 150.4 IU/mL). Within this concentration range, the system reached a reproducibility of 9–14%, measured as standard deviation what confirms the suitability of the sensor for the quantification of total IgE in human serum samples.

Regarding the specific IgE, the correlation between ImmunoCAP and the optoelectrical sensor was investigated using 385 IgE values to *A. alternate* (31), *A. vulgaris* (4), *C. arizonica* (19), *C. familiaris* (45), *D. farinae* (85), *D. pteronyssinus* (85), *F. silvestris* (44), Lipid transfer proteins (LTP) (11), *O. europaea* (35), and *P. Judaica* (26). Fig. 4B depicts the scatter diagram and regression line of inter-method comparison between the optoelectrical biosensor and ImmunoCAP for specific IgE. The good correlation obtained ($r = 0.99$; $P < 0.001$) reveals that both methods provide significantly similar quantitative information with regard the detection of specific IgE for the panel of allergens tested. Receiver Operating Characteristic (ROC) analysis and spearman correlations were performed for all allergens individually. Figs. S1 and S2 of the supplementary information depict the results. It is worth mentioning that all the positive results given by the ImmunoCAP method were corroborated. Indeed, excellent area under the curve (AUC) values were found, ranging from 0.882 to 1.0 for *D. pteronyssinus* and *A. vulgaris*, respectively. Indeed, the developed compact biosensor enables the simultaneous determination of specific IgE to a multitude of different allergens in a multiplex approach with only 25 μL of serum. This allows defining individual sensitization profiles from a single analysis that combined with patient's medical history might simplify identification of cross-reactivity and estimate the risk of severe reactions in cases of polysensitization. Besides, specific IgE, even in the absence of allergy, could be a risk factor for future clinical reactions or the memory of a previous allergic status (Alessandri et al., 2017).

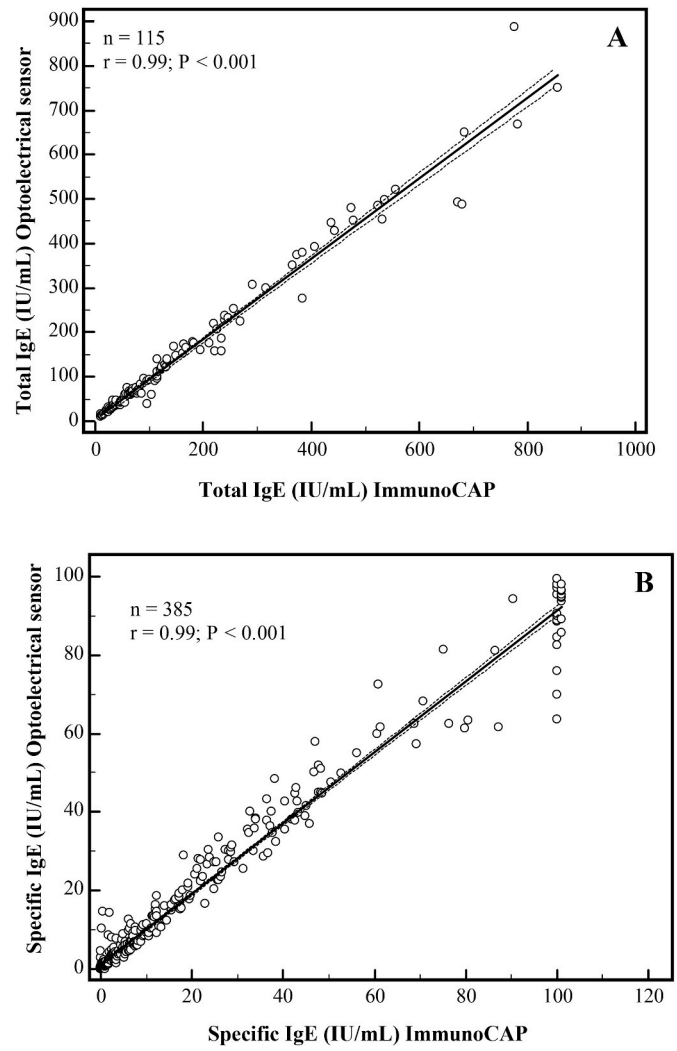

Fig. 4. Scatter diagram and regression line of inter-method comparison between the optoelectrical sensor and ImmunoCAP for total (A) and specific (B) IgE. Dotted line represents the 95% confidence interval.

As far as the ROC analysis is concerned for all ten different allergens evaluated together, an excellent area under the curve (AUC) value was found at 0.977 (Confidence interval, CI 0.957 to 0.990) compared to ImmunoCAP results (Fig. 5A). It is evident that the results closer to the upper left corners indicated that the prediction of both methods was highly comparable. Furthermore, the interactive dot diagram (Fig. 5B) reveals that the optoelectrical sensor reaches a sensitivity and specificity of 97.6 and 85.7, respectively, using 0.35 IU/mL as cut-off. Furthermore, the ability to correctly classify patients could be improved by using a threshold of 0.6 IU/mL, providing a clinical sensitivity and specificity of 94.9 and 94.0, respectively. These results confirm the suitability of the developed assay for allergy diagnosis.

Though the results are significantly similar between both methods, there are fundamental differences for measuring the concentration of allergen-specific IgE. In the ImmunoCAP system, there is a relative excess of allergen molecules immobilized in the CAP (1–2 μg) as compared to the amount of allergen immobilized on the DVD (0.5–1.0 ng), and with the quantity of allergen-specific IgE antibodies in serum. Other difference is the number of allergens simultaneously detected.

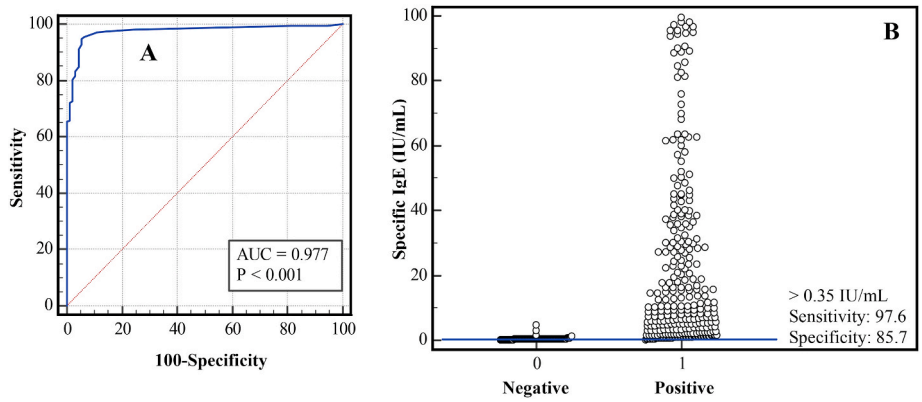

Fig. 5. Sensitivity and specificity of the optoelectrical sensor system as compared against ImmunoCAP for the ten different allergens (n = 385 values). A) ROC analysis representing the area under the curve (AUC). B) Interactive dot diagram. The horizontal line represents the cut-off threshold (0.35 IU/mL).

Here, as a proof of concept, our sensor system was designed to detect 12 allergens in a row. However, the versatility of the platform permits the implementation of other allergens on demand, increasing the number of samples per disc, as well. In this sense, the useful sensing surface rules the estimated capacity of the biosensors. In this report, the useful surface was ca. 66.5 cm^2 that is almost four times that of regular slides. Besides, taking into consideration that an array of 10 × 10 with spots of 500 μm in diameter each can fit 25 allergens with four replicates, the simultaneous detection of several hundreds of allergens (300–700) is estimated. As different array arrangements could be designed, our sensor would outperform the current multiplex assays. On the other hand, all the serum samples in this study were analysed, using 15 regular DVD-r; thus, from the economical point of view, as compared to ImmunoCAP system, the cost to detect specific IgE for 12 allergens (ca. 0.5 €) is at least 500 times less. Other major difference is the instrument required what makes the developed technology very interesting for primary care health and doctor office approaches. Furthermore, the system might be adapted to become semiautomatic, allowing the analysis of the samples using microfluidic discs (www.cobiophad.com). The estimated cost of our approach is below 500 € as compared to those large equipment such as ImmunoCAP, Immulite and fluorescent scanners (ISAC). The versatility of our approach was also demonstrated by measuring the specific IgG present in the samples analysed by ImmunoCAP. It is known that several mechanisms are involved in the immunotherapy-mediated desensitization to allergens, in which blocking antibodies IgG play a central role. Many studies have verified that after immunotherapy there is an increase of IgG specific antibodies (Demoly et al., 2017). In addition, the presence of specific IgG antibodies against an allergenic source, even though without allergic symptoms, could reflect a high allergen exposure. For these reasons, the detection and quantification of specific IgG antibodies is valuable in the control of immunotherapy progress and studies of exposure to allergens in a population. As the concentration of IgG against specific allergens might change depending on the area studied, sera from eight non-atopic individuals were used to stablish a threshold for each allergen. Table 1 shows the results. The wide range of threshold values (0.5–180.4 μg/mL) reveals the exposure to the different allergens in a geographical area. For example, the higher threshold observed for *A. alternata* (180.4 μg/mL) can be probably explained because this mold is ubiquitous in the region of Valencia, present indoors and outdoors, so the exposure is relatively high. On the other hand, the low IgG threshold value for LTP (0.5 μg/mL), reveals low exposure to these proteins in the non-atopic control group. Furthermore, the values were classified in four categories according to the IgG and IgE concentration. It was found that 76.4% of the values (class A plus B) were above 0.35 IU/mL (IgE positive), and 28.1% were above the IgG threshold (class A) for each allergen. Data showed a low number of values corresponding to class C (5.7%), as expected in a group of individuals who attended to the allergy specialist. Furthermore, 48.3% of the patients were grouped within class B, revealing the importance of the balance of these two immunoglobulins in the regulation of the disease in a cohort of allergic patients. Unfortunately, no information about exposure or administration of immunotherapy was recorded from the group of patients and controls analysed.

Table 1
Classification of patients according to IgG and IgE levels determined by the optoelectrical sensor and ImmunoCAP, respectively.

	CLASS				
IgG threshold (μg/mL)	A IgE+/ IgG+	B IgE+ / IgG-	C IgE- / IgG+	D IgE- / IgG-	Patient (n)
A. alternate (180.4)	14	4	4	9	31
A. vulgaris (6.4)	2	0	0	2	4
C. arizonica (32.9)	1	16	0	2	19
C. familiaris (20.5)	18	18	4	5	45
D. farina (14.7)	17	49	4	15	85
D. pteronyssinus (28.5)	20	47	3	15	85
F. silvestris (49.3)	4	30	1	9	44
Grass pollen (9.0)	0	0	0	0	0
LTP (0.5)	8	1	0	2	11
O. europea (66.5)	7	19	1	8	35
P. judaica (2.2)	17	2	5	2	26
Profilin (10.7)	0	0	0	0	0
Total values	**108**	**186**	**22**	**69**	**385**
(%)	28.1	48.3	5.7	17.9	

4. Conclusions

One of the main benefits of our optoelectrical biosensor for allergy diagnosis is its good performances such as versatility, cost-effectiveness, high sensitivity and specificity that make the system a component-resolved diagnostic method capable to determine simultaneously total and specific IgE and IgG against individual allergen molecules or components on demand. In addition, the obtained analytical results are comparable to the reference method. In fact, herein-described biosensor system might provide relevant information on the sensitization profile to extracts and purified native or recombinant allergens within the same assay. In this context, 12 allergens (extracts and pure proteins), as proof of concept, immobilized on a regular DVD, simplified the evaluation of sensitization profiles. Besides, the use of allergen extracts containing all the major and minor allergenic components in combination with pure allergens might influence positively in the analytical and clinical performances of the biosensor. Indeed, it might be expected a significantly improvement of the analytical performances of the optoelectrical sensor as the number of immobilized components increases.

Considering the global social and personal costs, the availability of the results in a single assay has clear additional benefits. This seems particularly interesting considering that the gross cost of testing 20 samples against a panel of 12 allergens on the DVD is approximately 0.50 € what makes it very affordable to every laboratory.

The advantage of having a versatile biosensor system with the ability to expand the repertoire of components on demand will facilitate an evaluation of sensitization profiles against other allergens (indoor, outdoor, food, and drug allergens), which is rarely carried out with current *in vitro* tests, mainly due to its high cost. This plus might allow the allergist to accurately informing allergen-avoidance regimes, and consequently to better design personalized therapeutic strategies.

Future perspectives relates with multiplexed analysis of allergic and non-allergic individuals over time, thinking on the ability of artificial intelligence to manage huge information and generate mathematical predictive models for obtaining of accurate deductions and predictions. This could facilitate the elucidation of the medical history and prevention of new allergies, reducing health-care costs. Indeed, the described system fulfils the requirements of a cost-effective multiplexed biosensor system for point-of care applications. The analytical system can be used completely autonomously in clinical or mobile laboratory settings without the need for any additional medical equipment, which could make it suitable for massive allergy screening campaigns. In summary, the system here described is prompt to detect specific IgE and IgG against individual allergen molecules or components using purified native or recombinant allergens, providing further diagnostic information in patients with any type of IgE-mediated allergy in terms of predicting clinical relevance or prognosis. The system meets all the requirements in terms of sensitivity, specificity and versatility to perform immunoassays directly from whole blood in a lab-on-a-disk format. Besides, the detection limit could be improved by working in two ways. On one hand, increasing the total assay time would result in a better detection limit. On the other hand, including a wide spectrum of allergens to better cover the identification of new sensitization profiles could improve the analytical performances as well. In addition, the full automation of the optoelectrical biosensor system for IgE mediated allergy diagnostics with sample-in-answer-out capability to improve patients care is envisioned.

CRediT authorship contribution statement

Salvador Mas: Investigation, Methodology, Writing - original draft, Validation. **Ahmed A. Badran:** Investigation, Methodology, Writing - original draft, Validation. **María-José Juárez:** Software, Validation. **Dolores Hernández Fernández de Rojas:** Conceptualization, Resources. **Sergi Morais:** Conceptualization, Supervision, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Ángel Maquieira:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was granted by the Spanish Ministry of Economy and Competitiveness (CTQ2016-75749-R), FEDER and by the Ayudas UPV-LA FE 2019 (P105 VALBIOAL). Thanks to the Servicio de Alergia del Hospital Universitari i Politènic La Fe for providing human serum samples. M.J.J. and A.A. Badran gratefully acknowledges the fellowships to carry out the Ph. D studies funded by Universitat Politecnica de Valencia (programa para la formación de personal investigador (FPI, 2017) and Generalitat Valenciana (programa Santiago Grisolía: 2013/040), respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112438.

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Highly sensitive optoelectrical biosensor for multiplex allergy diagnosis

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Supplementary material

Figure S1. ROC curves for the tested allergens. 2

Figure S2. Scatter and linear regression for the tested allergens 3

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Figure S1. ROC curves for the tested allergens.

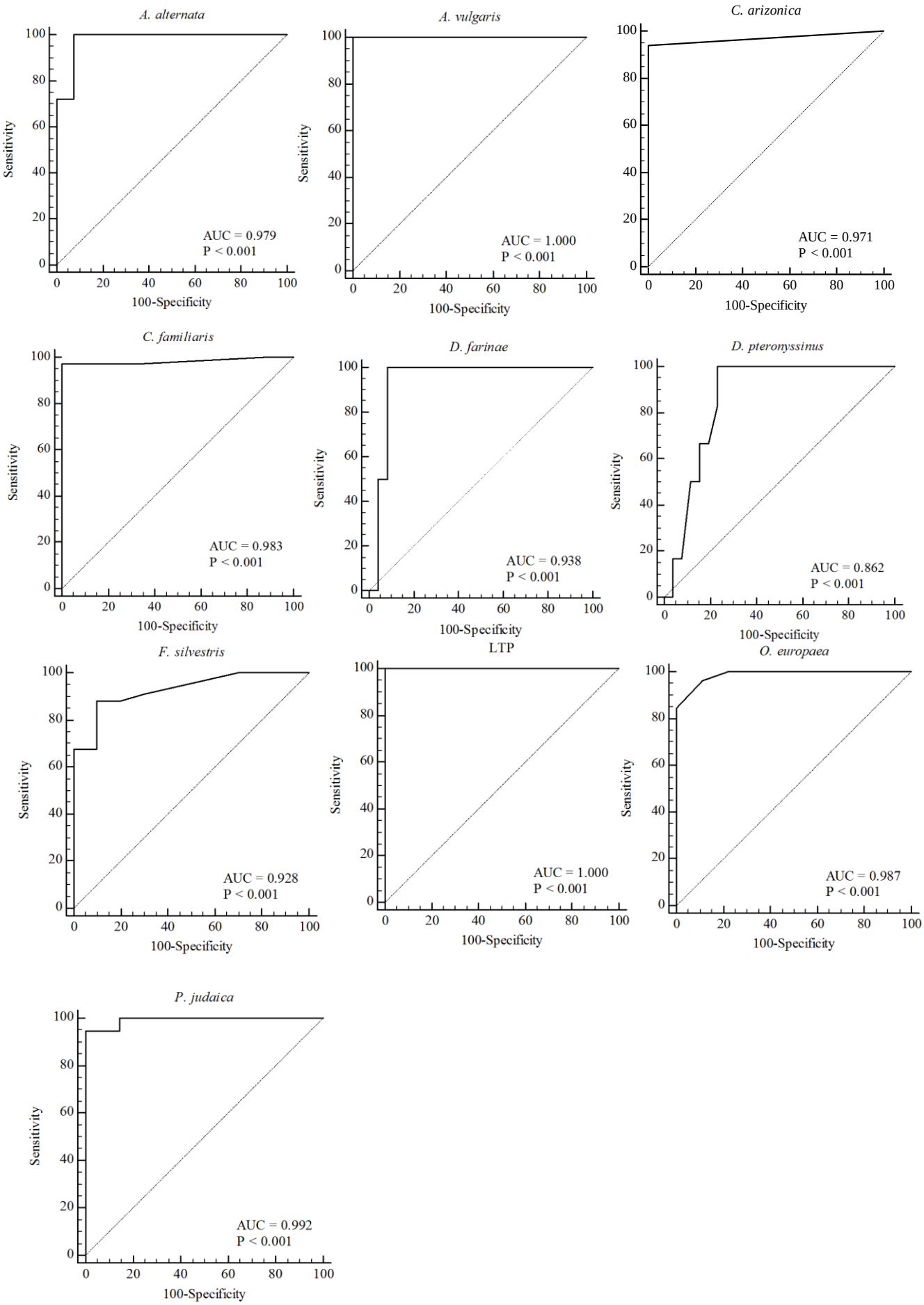
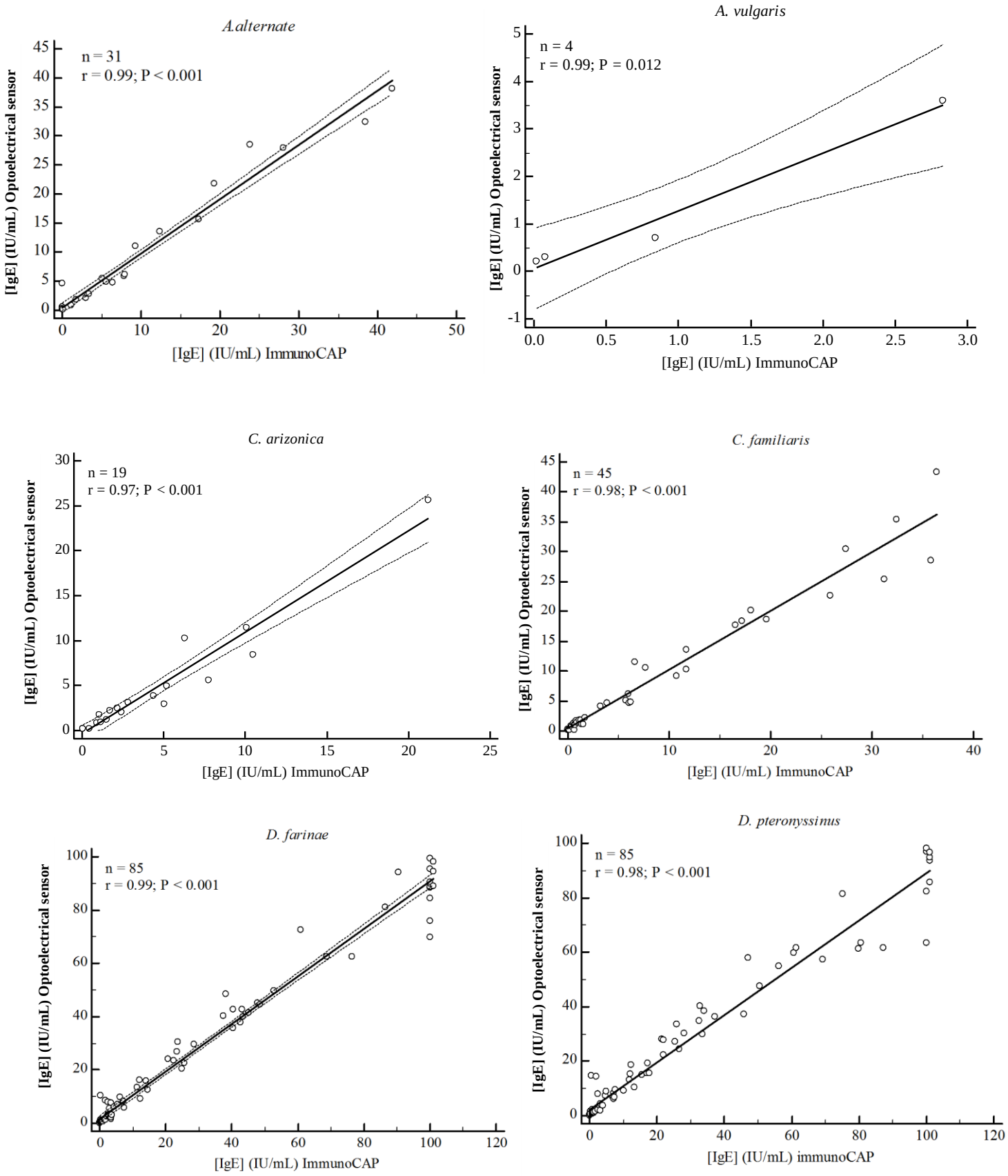
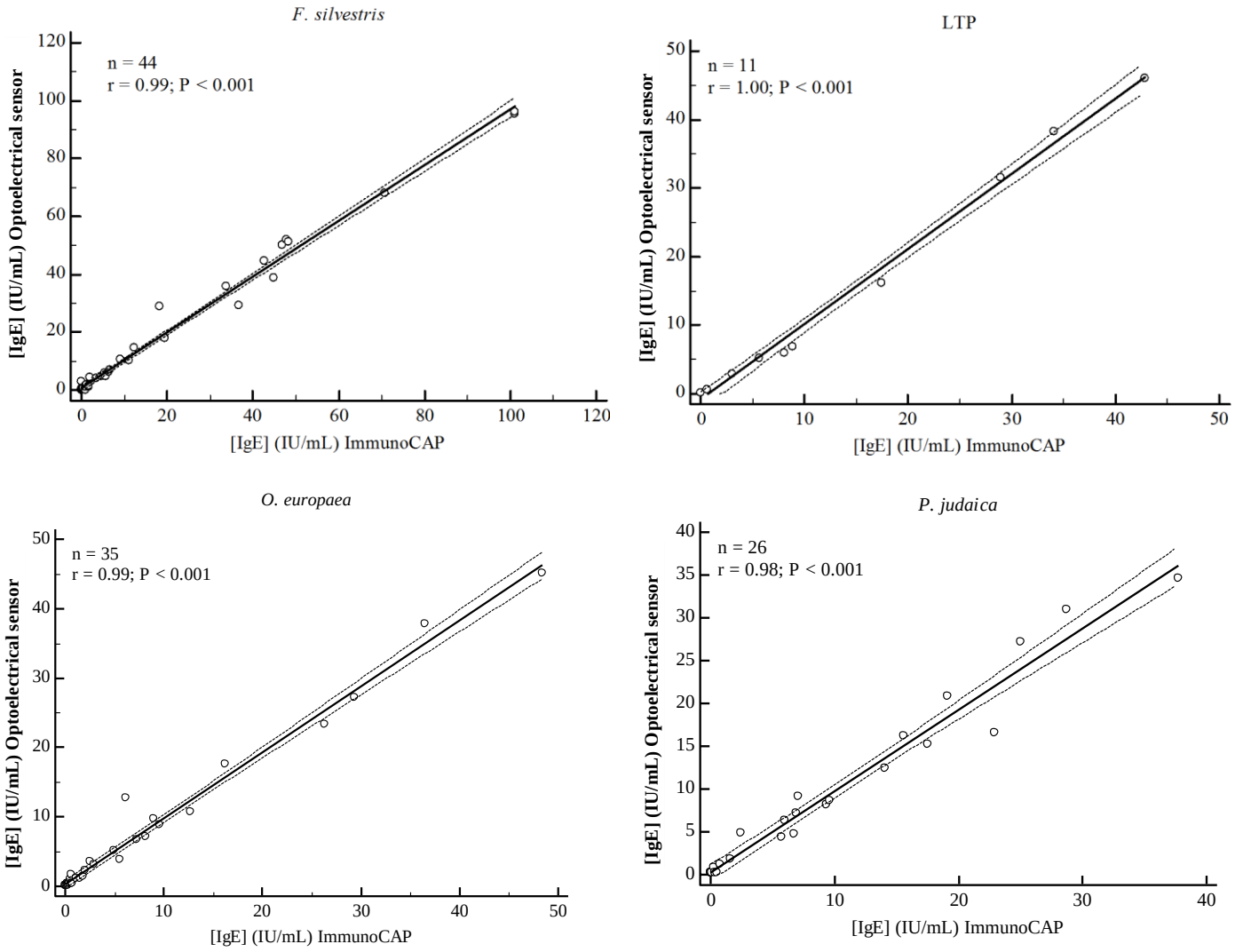


Figure S2. Scatter and linear regression for the tested allergens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