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Validation of a commercial allergen microarray platform for specific immunoglobulin E detection of respiratory and plant food allergens

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ABSTRACT

Background: As the use of multiplex-specific immunoglobulin E (sIgE) detection methods becomes increasingly widespread, proper comparative validation assessments of emerging new platforms are vital.
Objective: To evaluate the clinical and technical performance of a newly introduced microarray platform, Allergy Explorer (ALEX) (MacroArray Diagnostics), in the diagnosis of pollen (cypress, grass, olive), dust mite (*Dermatophagoides pteronyssinus*), mold (*Alternaria alternata*), fruit (apple, peach), and nut (walnut, hazelnut and peanut) allergies and to compare it with those of the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) 112 microarray and the ImmunoCAP singleplex method (ThermoFisher Scientific).
Methods: We enrolled 153 patients with allergy and 16 controls without atopy. The sIgE assays were conducted using ISAC112, ALEX version 2 (ALEX2), and ImmunoCAP for whole extracts and major components. Technical validation of ALEX2 was performed by measuring repeatability and interassay, interbatch, and interlaboratory reproducibility.
Results: When measured globally (detection by 1 or more allergen components), ALEX2 had adequate sensitivity and specificity for most of the allergens studied, comparable in general with that of ISAC112 (except for olive pollen and walnut) and similar to that of ImmunoCAP whole extract measurements. Component-by-component analysis revealed comparable results for all techniques, except for Ole e 1 and Jug r 3, in both ISAC112 and ImmunoCAP comparisons, and Alt a 1, when compared with ISAC112. Continuous sIgE levels correlate with sIgE by ImmunoCAP. Good reproducibility and repeatability were observed for ALEX2.
Conclusion: ALEX2 has sound technical performance and adequate diagnostic capacity, comparable in general with that of ISAC112 and ImmunoCAP.

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Introduction

The use of multiplex platforms in component-resolved diagnosis has aided allergists in obtaining detailed information on a patients' specific allergenic profile by means of a single-measurement method. Nevertheless, some limitations have been uncovered for the first commercially available allergen platforms.[1,2] Furthermore, adequate panel selection[3,4] and the inclusion of whole-extract detection as a complement to component analysis were found to be essential for an adequate and cost-effective diagnosis.[5,6] A new microarrayed allergen platform, named Allergy Explorer (ALEX, MacroArray Diagnostics, Vienna, Austria), has recently been introduced. By means of nanoparticle-based technology, it performs a quantitative analysis of specific immunoglobulin E (sIgE) to a combination of natural and recombinant allergen components, including whole extracts. Its diagnostic capacity seems to correlate well with that of 2 previously established techniques, the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC)[7-9] and ImmunoCAP singleplex method.[9-11]

This study aims to evaluate the clinical performance of ALEX version 2 (ALEX2) in the diagnosis of pollen (cypress, grass, olive), dust mite (*Dermatophagoides pteronyssinus*), mold (*Alternaria alternata*), fruit (apple, peach), and nut (walnut, hazelnut and peanut) allergies. We also sought to compare its diagnostic capacity with the ImmunoCAP ISAC 112 microarray (ISAC112) and ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden). Finally, we aimed to perform a technical validation studying the repeatability, reproducibility, and quantification capacity of ALEX2 using linearity assessment.

Methods

Patients

We included 169 subjects: 153 patients with allergy to at least one of the following allergenic biological sources: cypress, grass, olive pollen, *D pteronyssinus, A alternata*, apple, peach, walnut, hazelnut and peanut; and 16 controls without atopy. Most patients (77%) were selected from a previous multicentric study (grant: PI 11/01634) that evaluated the diagnostic performance of the ISAC112 microarray (ThermoFisher Scientific) in a sample from 14 hospitals from different areas in Spain.[1,3,12] New eligible patients were recruited at Clínica Universidad de Navarra in Pamplona, Navarra (Spain), for dust mite or nut allergy, and at Hospital Clínic in Barcelona (Spain) for *Alternaria* allergy. Inclusion and exclusion criteria are detailed in eMethods. The study protocol was approved by the Ethics Committee of the coordinating center (045/2011), and all participants provided written informed consent.

Multiplex-Specific Immunoglobulin E Assay

All sera were tested using ALEX2 (44 allergens) and ISAC112 (33 allergens) for the 10 sources evaluated (eTable 1). Total and sIgE against the described allergens were evaluated using the ALEX2 platform according to manufacturer's instructions (detailed in eMethods). The Raptor software (MacroArray Diagnostics) calculates the levels of immunoglobulin (Ig)E based on the intensity of precipitated color in the membrane according to a calibration curve measured in kilounits per liter for values between 0.30 and 50 kU_A/L. Total IgE is also measured for values between 20 and 2500 kU/L. Values of sIgE equal to or greater than 0.30 kU_A/L were considered positive, following manufacturers' instructions. For clinical validation, ALEX2 was performed using the automatic robot Madmax (MacroArray Diagnostics).

Specific IgE values against the described allergens (eTable 1) were measured in all patients using ISAC112 (ThermoFisher), according to manufacturer's instructions (detailed in eMethods). Specific IgE values are expressed semiquantitatively as ISAC Standard Unit (ISU). Results equal to or greater than 0.30 ISU were considered positive, per manufacturer's instructions.

Singleplex Specific Immunoglobulin E Assay

For each evaluated biological source and for both cases and controls, sIgE levels to the whole extract and major allergen components in our area (eTable 1) were determined by fluorescence enzyme immunoassay (ImmunoCAP, ThermoFisher). Cup a 1 was detected to be the major allergen of *Cupressus arizonica*, as were Phl p 1, Ole e 1, Der p 1 and Der p 2, Alt a 1, Mal d 3, Pru p 3, Jug r 3, Cor a 8, and Ara h 9 as major allergens of *Phleum pratense, Olea europea, D pteronyssinus, A alternata*, apple, peach, walnut, hazelnut, and peanut, respectively (based on previous studies).[1,3] In addition, Der p 23 was measured for *D pteronyssinus* cases and controls. Specific IgE values equal to or higher than 0.35 kU_A/L by fluorescence enzyme immunoassay were considered positive.

Technical Validation

Repeatability was evaluated using intra-assay analysis, and reproducibility was evaluated using interassay, interbatch, and interlaboratory analyses for the 44 evaluated allergens (eTable 1). ALEX2 repeatability was tested by analyzing a pool of sera in 10 measurements performed in the same assay, with the same ALEX2 kit (same batch), in 1 laboratory. Reproducibility of the platform was evaluated by analyzing the interassay sIgE variability from 10 samples (9 individual sera and the pool) and by repeating the ALEX2 technique in 5 different days, using chips from the same production batch, in the same laboratory. Interbatch reproducibility was also evaluated by analyzing sIgE levels from the 10 samples using 2 different chips and reagents from different production batches. Finally, interlaboratory reproducibility was studied by analyzing the levels of sIgE obtained from the 10 samples using chips from the same batch (reagents from different batches) analyzed in the following 2 different laboratories: Clínica Universidad de Navarra (Pamplona, Spain) and Hospital Clínic (Barcelona, Spain). The reading of the chips was performed in each laboratory according to manufacturer's instructions.

To explore quantitative sIgE determination capacity of ALEX2, serial dilutions of several sera were analyzed. ALEX2 test was performed on the same day, using the same batch in the same laboratory, using the pool of sera and 3 sera as described: concentrated, 1:2, 1:5, and 1:10, diluted in water. ALEX2 technique was performed manually for technical validation and blocking cross-reactive carbohydrate determinant interference according to manufacturer's instructions.

Statistical Analysis

Variables were evaluated for normal distribution (Shapiro-Wilk test). Nonnormal quantitative values were described as medians and interquartile ranges (25-75 percentile). Qualitative values were described as frequencies (percentages). Proportions were compared using the χ^2 test or Fisher exact test. Ordinal and quantitative variables were analyzed using the *U* Mann-Whitney test. Qualitative results comparing performance of 2 techniques for the same sample were analyzed by McNemar's test. Concordance between variables was analyzed using the Kappa index (κ) and was interpreted to be poor (<0.2), weak (0.21-0.4), moderate (0.41-0.6), good (0.61-0.8), or very good (0.8-1) according to the Altman model.[13] The Wilcoxon signed rank test was used for quantitative comparison in paired samples. The correlation between the quantitative variables was evaluated using the Spearman correlation coefficient. Clinical statistical analysis was performed using Stata 12.0 (StataCorp). Differences of *P* less than .05 were statistically significant.

Table 1
Se and Sp of sIgE in the Diagnosis of Allergy Using ALEX2, ISAC112, and ImmunoCAP

Biological source		ALEX2			ISAC112		ALEX2 vs ISAC112 McNemmar test *P*		Whole extract ImmunoCAP		ALEX2 vs Immuno CAP McNemmar test *P*
		Se	Sp		Se	Sp			Se	Sp	
Cypress pollen	Cup a 1	85.7	96.2	Cup a 1	85.7	92	>.99	Cup a	82.1	96.2	>.99
Grass pollen	Phl p 1, 2, 5.0101, 6, 7, 12	100	88.9	Phl p 1, 2, 4, 5, 6, 7, 11, 12	96.5	88.9	>.99	Phl p	96.4	92.3	.50
Olive pollen	Ole e 1, 7, 9	55.5	96.8	Ole e 1, 7, 9	85.2	90.3	**.002**	Ole e	92.6	87.1	**<.001**
Dust mite	Der p 1, 2, 5, 7, 10, 11, 20, 21, 23	95	100	Der p 1, 2, 10	80	100	.25	Der p	85	100	.62
Alternaria	Alt a 1, 6	88.9	100	Alt a 1, 6	55.6	100	**.03**	Alt a	—[a]	100	.50
Apple	Mal d 1, 2, 3	73.3	100	Mal d 1	6.7	100	**.002**	Mal d	100	100	.12
Peach	Pru p 3, 7	94.1	100	Pru p 1, 3, 4	97.1	100	>.99	Pru p	100	100	.50
Walnut	Jug r 1, 2, 3, 4, 6	60.7	100	Jug r 1, 2, 3	85.7	95.7	**.02**	Jug r	92.9	100	**.01**
Hazelnut	Cor a 1.0401, 8, 9, 11, 12, 14	100	91.3	Cor a 1.0104, 8, 9	90	100	.12	Cor a	100	100	.50
Peanut	Ara h 1, 2, 3, 6, 8, 9, 15	94.7	95.7	Ara h 1, 2, 3, 6, 8, 9	84.2	100	.25	Ara h	100	100	>.99

Abbreviations: ALEX2, Allergy Explorer version 2; ISAC112, Immuno Solid-phase Allergen Chip 112; Se, sensitivity; sIgE, specific immunoglobulin E; Sp, specificity.
NOTE. Positivity to all or 1 of the components representing the biological source was regarded as diagnostic for ALEX2 and ISAC112, whereas positivity to whole extract was considered for ImmunoCAP. *P* values <.05 are marked in bold.
[a]Positive sIgE to *Alternaria alternata* by ImmunoCAP was an inclusion criterion for *Alternaria* cases, and thus Se has not been calculated.

We also analyzed the strength of agreement of the results obtained for all 44 allergens. Repeatability of these results under the same conditions was evaluated by calculating the global intraclass coefficient correlation (ICC), and reproducibility of the results obtained in the different assays (interassay, interbatch, and interlaboratory) under different conditions was evaluated by calculating the ICC for each allergen. The level of agreement using the ICC was expressed using the Fleiss classification[14]: very good (ICC > 0.90), good (0.71-0.90), moderate (0.51-070), mediocre (0.31-0.50), or poor (ICC < 0.30). For the pooled sera, sIgE values to the studied allergens were also analyzed individually by calculating the coefficient of variation (CV) for positive results (allergens revealing positive results in at least 50% of the 10 repeated measurements) in the intra-assay and interassay analyses (for allergens revealing positive results in at least 50% of the 5 repeated measurements). Furthermore, we analyzed the reliability of the technique comparing the number of allergens that modified the positivity vs negativity of the IgE according to the range established by the manufacturer (0.3 kUA/L) in the intra-assay, interassay, interbatch, and interlaboratory measurements for each serum. The ICC for each allergen was performed using the statistical software package Statistical Package for Social Science for Windows, version 20 (IBM). The CV was calculated using Excel 2016, Microsoft Office (Microsoft). Linearity was evaluated by analyzing slope and R^2 with simple linear regression and comparing serial diluted sIgE data with expected values for all allergens having basal results equal to or above 3 kUA/L. Linearity analysis and graphs were performed in GraphPad Prism 9.0 (GraphPad Software).

Results

Patient Characteristics

From 153 patients with allergy and 16 controls without atopy, the cases and controls were selected for each allergen using the specified criteria. Thus, for the aeroallergen diagnostic performance analysis of the microarray, we included 28 cases and 26 controls (50% without atopy) for cypress pollen, 29 cases and 27 controls (48% without atopy) for grass pollen, 27 cases and 31 controls (52% without atopy) for olive pollen, 20 cases and 22 controls (59% without atopy) for *D pteronyssinus*, and 18 cases and 22 controls (45% without atopy) for *A alternata*. For food allergens, apple was represented by 15 cases, peach by 34, walnut by 28, hazelnut by 20, and peanut by 19 cases, together with 23 controls (69.6% without atopy). Clinical and demographic data of the individuals included in the study, grouped into cases and controls, are summarized for allergy diagnosis to respiratory allergens ineTable 2A and to food allergens in eTable 2B.

Global Diagnostic Performance of the Allergy Explorer Version2 Microarray Is Adequate for Most of the Studied Allergens

When considering positivity of 1 or all components representing the evaluated biological source as diagnostic of allergy, ALEX2 yielded good performance for cypress pollen, grass, *D pteronyssinus, A alternata*, apple, peach, hazelnut, and peanut. Improvable sensitivity (Se) was observed for olive pollen and walnut allergy (Table 1). Olive pollen's Se was increased when Fra e 1, the major allergen of the Oleaceae ash pollen, was considered in the calculations (Se: 77.8%, specificity [Sp]: 93.5%). In fact, higher levels of sIgE to Fra e 1 (3.07 [0.48-19.23] kU_A/L) than for Ole e 1 (0.76 [0-5.47] kU_A/L) were observed in olive pollen cases (Wilcoxon test $P < .001$) by ALEX2, with 81.5% of patients with allergy to olive being from areas without ash pollen-relevant levels.

Table 2
Frequency of Sensitization to the Components From the Studied Sources Present in ALEX2, ISAC112, and ImmunoCAP, for Cases and Controls

% Sensitization		ALEX2		ImmunoCAP		ALEX2 vs ImmunoCAP	ISAC112		ALEX2 vs ISAC112
Biological source and components		Cases	Controls	Cases	Controls	κ (*P* McNemmar)	Cases	Controls	κ (*P* McNemmar)
Cypress pollen	Cup a 1	85.7	3.8	82.1	3.8	0.963 (>.99)	85.7	8	0.962 (>.99)
Grass pollen	Phl p 1	96.6	11.1	96.4	7.1	0.893 (.25)	93.1	3.7	0.928 (.5)
	Phl p 2	48.3	3.7				37.9	3.7	0.854 (.25)
		Phl p 5.0101					Phl p 5		
	Phl p 5	37.9	3.7				34.5	3.7	0.945 (>.99)
	Phl p 6	34.5	3.7				24.1	3.7	0.811 (.25)
	Phl p 7	6.9	3.7				3.4	3.7	0.811 (>.99)
	Phl p 12	17.2	0				13.8	3.7	0.780 (>.99)
Olive pollen	Ole e 1	55.6	3.2	66.7	6.5	0.839 (.12)	74.1	9.7	**0.734 (.01)**
	Ole e 7	0	0				22.2	0	**0.000 (<.001)**
	Ole e 9	3.7	0				0	0	0.000 (>.99)
Dermatophagoides pteronyssinus	Der p 1	60	0	70	0	0.889 (.5)	60	0	1 (>.99)
	Der p 2	70	0	70	0	1 (>.99)	65	0	0.946 (>.99)
	Der p 5	40	0						
	Der p 7	55	0						
	Der p 10	0	0				10	0	0.000 (.5)
	Der p 11	0	0						
	Der p 20	0	0						
	Der p 21	35	0						
	Der p 23	85	0	70	4.7	0.842 (>.99)			
Alternaria	Alt a 1	88.9	0	100	0	1 (>.99)	55.6	0	**0.6667 (.03)**
	Alt a 6	0	0				11.1	0	0.000 (.5)
Apple	Mal d 1	6.7	0				6.7	0	0.493 (>.99)
	Mal d 2	0	0						
	Mal d 3	66.7	0	86.7	0	0.691 (.37)			
Peach	Pru p 1						0	0	
	Pru p 3	94.1	0	96.9	4.3	0.927 (.5)	97.1	0	0.893 (>.99)
	Pru p 7	0	0						
Walnut	Jug r 1	14.3	0				14.3	0	1 (>.99)
	Jug r 2	17.9	0				17.9	4.4	0.694 (>.99)
	Jug r 3	46.4	0	64.3	0	0.771 (.06)	67.9	0	0.642 (.07)
	Jug r 4	10.7	0						
	Jug r 6	14.3	0						
Hazelnut	Cor a 1.0401	5	4.3				5	0	0.656 (>.99)
	Cor a 8	90	0	90	0	1 (>.99)	80	0	0.903 (.5)
	Cor a 9	5	0				5	0	1 (>.99)
	Cor a 11	5	4.3						
	Cor a 12	0	0						
	Cor a 14	10	0						
Peanut	Ara h 1	15.8	0				15.8	0	1 (>.99)
	Ara h 2	15.8	0				15.8	0	1 (>.99)
	Ara h 3	10.5	0				5.3	0	0.656 (>.99)
	Ara h 6	15.8	0				15.8	0	0.641 (>.99)
	Ara h 8	0	0				0	0	NA
	Ara h 9	84.2	0	84.2	0	1 (>.99)	73.7	0	0.897 (.5)
	Ara h 15	0	4.3						

Abbreviations: ALEX2, Allergy Explorer version 2; ISAC112, Immuno Solid-phase Allergen Chip 112; NA, not analyzed; sIgE, specific immunoglobulin E.
NOTE. Qualitative comparisons are represented in terms of positive/negative results between ALEX2 sIgE and ImmunoCAP/ISAC112 sIgE to different components. Negative results by all techniques were not analyzed. *P* values < .05 are marked in bold.

Diagnostic Performance of the Allergy Explorer Version 2 Microarray Is Quite Similar to Immuno Solid-phase Allergen Chip Microarray for the Evaluated Allergens

Component-based sensitization profiles in cases and controls were also evaluated using the ISAC112 multiplex platform for the selected allergens (Table 2). The global diagnostic capacity of ALEX2 was compared with that observed for ISAC112, considering the components present there for the same evaluated biological sources. Sensitivity and specificity for both platforms are presented in Table 1. ISAC112 was found to have comparable performance with ALEX2 for cypress pollen, grass pollen, *D pteronyssinus*, peach, hazelnut, and peanut allergy detection. ISAC112 Se was found to be higher for the detection of olive pollen (P = .002) and walnut (P = .02) allergies, whereas it was significantly lower for the detection of *Alternaria* (P = .03) and apple (P = .002) allergies.

When analyzing data on a component-by-component basis for those elements common to both the ALEX2 and ISAC112 platforms, ALEX2 was found to have a similar diagnostic performance compared with ISAC112 in all of them except for Ole e 1, Alt a 1, and the under-researched allergen Ole e 7; Jug r 3 tended to have a worse performance by ALEX2 than ISAC112, but differences did not reach statistical significance. Comparative data are found in Table 2.

Diagnostic Performance of Allergy Explorer Version2 Microarray Is Quite Similar to ImmunoCAP for Whole Extract and Components of the Evaluated Allergens

The sIgE levels to whole extracts and to major allergens of the evaluated allergenic sources were also measured using the singleplex ImmunoCAP. The overall diagnostic capacity of ALEX2 was compared with that found for the selected sources of whole extracts by

Table 3
sIgE Levels (kU_A/L) Against the Major Allergens Measured by ALEX2 and by ImmunoCAP, for Cases and Controls

		ALEX2 kU_A/L, median (IQR)		ImmunoCAP kU_A/L, median (IQR)		ALEX2 vs ImmunoCAP Spearman correlation	
Biological source		Cases	Controls	Cases	Controls	*Rho*	*P*
Cypress pollen	Cup a 1	7.45 (1.39-17.33)	0.05 (0-0.12)	3.84[a] (0.82-9.88)	0[a] (0-0.02)	0.890	<.001
Grass pollen	Phl p 1	6.72 (4.35-23.2)	0 (0-0.06)	4.01[a] (2.03-9.55)	0 (0-0)	0.915	<.001
Olive pollen	Ole e 1	0.76 (0-5.47)	0 (0-0)	0.67[b] (0.08-7.74)	0[b] (0-0.03)	0.895	<.001
Dermatophagoides pteronyssinus	Der p 1	3.3 (0-9.45)	0 (0-0)	2.33 (0.02-10.65)	0 (0-0)	0.856	<.001
	Der p 2	21.25 (0.15-33.43)	0.03 (0-0.08)	7.19[a] (0.04-18.5)	0[a] (0-0)	0.853	<.001
	Der p 23	5.39 (1.13-10.4)	0 (0-0)	1.47[a] (0.17-4.97)	0[b] (0-0.02)	0.810	<.001
Alternaria alternata	Alt a 1	28.05 (8.86-36.03)	0 (0-0)	8.59[a] (2.59-12.5)	0 (0-0)	0.944	<.001
Apple	Mal d 3	1.79 (0.09-7.95)	0 (0-0)	2.73 (1.2-3.95)	0[b] (0-0)	0.834	<.001
Peach	Pru p 3	5.39 (2.55-15.64)	0 (0-0)	6.17 (2.35-12.2)	0[b] (0-0)	0.928	<.001
Walnut	Jug r 3	0.17 (0.01-2.16)	0 (0-0.01)	1.03 (0.1-2.38)	0.01[b] (0-0.02)	0.799	<.001
Hazelnut	Cor a 8	2.09 (1.41-9.09)	0.06 (0.03-0.14)	1.19[a] (0.66-2.7)	0[a] (0-0)	0.824	<.001
Peanut	Ara h 9	3.93 (2.28-10.99)	0.1 (0.02-0.73)	2.29[a] (1.23-6.23)	0[a] (0-0.01)	0.780	<.001

Abbreviations: ALEX2, Allergy Explorer version 2; IQR, interquartile range; sIgE, specific immunoglobulin E.
NOTE. Median and IQR are presented. Spearman's correlation between the 2 techniques was calculated combining cases and controls together. Spearman's correlation rho coefficients and *P* values are presented.
[a]Wilcoxon signed rank test at $P < .05$ between sIgE by ALEX2 and ImmunoCAP revealing higher sIgE by ALEX2 than by ImmunoCAP for cases and controls.
[b]Wilcoxon signed rank test at $P < .05$ between sIgE by ALEX2 and ImmunoCAP revealing higher sIgE by ImmunoCAP than by ALEX2 for cases and controls.

ImmunoCAP (Table 1). Similar diagnostic performance was observed for most of the evaluated allergens (cypress pollen, grass pollen, *D pteronyssinus, A alternata*, apple, peach, hazelnut, and peanut). Se for ImmunoCAP whole extract was significantly superior than that for ALEX2 in the diagnosis of olive pollen ($P < .001$) and walnut ($P = .01$) allergy. Se for sIgE to *A alternata* by ImmunoCAP was not compared with ALEX2, because our inclusion criteria included positivity of the *A alternata* whole extract allergen when considering patients as "Alternaria allergic."

Moreover, when analyzing data on a component-by-component basis for selected allergens (major allergens in our sample) by ALEX2 and ImmunoCAP, ALEX2 was found to have similar diagnostic performance for all the allergens tested; Jug r 3 tends to have worse performance by ALEX2 than by ImmunoCAP, but these differences do not reach statistical significance (Table 2). In addition, in the knowledge that the ALEX2 platform has been created as a quantitative method, correlation analysis was performed using sIgE against selected major components between ImmunoCAP and ALEX2, revealing high correlation coefficients, all of them more than 0.750. Given that ImmunoCAP and ALEX2 results are both measured in kU_A/L, quantitative comparisons were performed for sIgE to these major components resulting in higher sIgE levels by ALEX2 than by ImmunoCAP for 7 of the 12 allergens in cases and higher sIgE by ImmunoCAP than ALEX2 in 7 of the 12 allergens in controls (Table 3).

Total Immunoglobulin E

Taking into consideration that total IgE is measured semiquantitatively by ALEX2, a quantitative correlation analysis was performed for total IgE measured by ALEX2 and ImmunoCAP. Total IgE values obtained in ALEX2 below 20 and above 2500 kU/L were not included, because the platform cannot detect them precisely, and neither were total IgE values by ImmunoCAP below 2 and above 5000 kU/L, according to manufacturer's specifications. Thus, 163 patients (147 cases and 16 controls) were finally used for the analysis. A good correlation was observed between both techniques (Spearman's rho: 0.8114; $P < .001$) (eFig 1).

Technical Validation

The global repeatability of the ALEX2 array was found to be excellent, 0.9979 (Cronbach's alpha). Variability of the repeated measurements for the studied allergens (intra-assay analysis) was also analyzed individually by calculating the coefficient of variation (CV) for positive results (allergens with a median value ≥ 0.3 kU_A/L: 32 of 44 allergens) for the pooled sera. CV data were grouped in ranges on the basis of sIgE levels of each allergen, and median intra-assay CV were calculated for each range (Table 4). All allergens with values more than 1 kU_A/L had CVs under 15%, whereas higher CVs were observed for values between 0.3 and 1. As the group size for this range was very small, we also analyzed its CV using all allergens in the array which revealed positive sIgE between 0.3 and 1 kU_A/L (N = 50). When doing so, the actual median CV was 20.8% (13.7%-27.9% interquartile range). Finally, when considering all 44 allergens, repetitions were consistent for all of them in terms of sensitization results (positive vs negative) except for Alt a 6, for which half of the results were positive and half were negative, have a high variability (CV: 107%, median, range: 0.29, 0.10-1.66 kU_A/L) and Der p 21, which revealed only 1 result above 0.30 kU_A/L (CV: 100%, median, range: 0.09, 0.00-0.31 kU_A/L). Two other allergens which had positive and negative results in the repeated measurements presented moderate variability and data scattered on the positivity cutoff values: Phl p 5.0101 (CV: 29%, median, range: 0.37, 0.21-0.55 kU_A/L) and Phl p 6 (CV: 38%, median, range: 0.41, 0.23-0.85 kU_A/L).

Reproducibility was evaluated individually for the studied allergens using the following 2 approaches: the CV calculated for positive results in the interassay analysis (allergens with a median value ≥ 0.3 kU_A/L: 33 of 44) for the pooled sera and ICC for 10 different samples

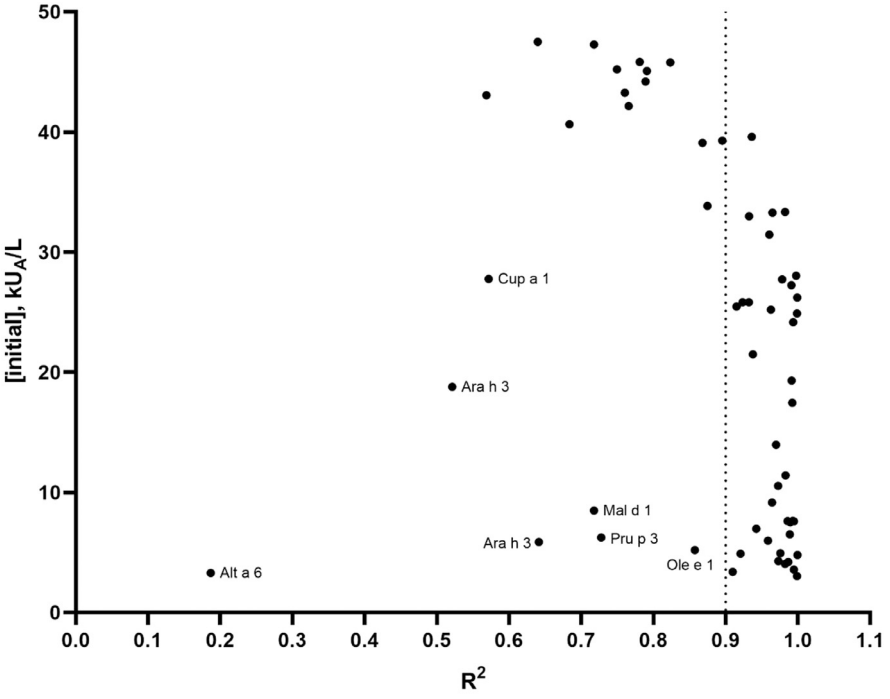

Figure 1. Graphical presentation of the linearity analysis in 4 samples, covering 28 of 44 studied allergens (59 determinations). The graph plots R^2 values (from linear regression analysis with the serial dilutions) (X-axis) and allergen initial sIgE concentrations (Y-axis). Allergens with an initial concentration less than 30 kU_A/L and a R^2 less than 0.9 are identified. sIgE, serum immunoglobulin E.

(pooled sera and 9 samples). CV was grouped on the basis of sIgE ranges of sIgE levels, and median interassay CV was calculated for each range (Table 4). As expected, slightly higher variability was observed for pooled sera measurements conducted in different days, compared with repetitions in the same day, but median CV for allergens with values over 1 kU_A/L was also under 15% (Table 5). Once more, when evaluating the CV in the low range (0.3-1 kU_A/L), we had a small group size, and thus, we also analyzed it with all eligible allergens in the array (N = 48) to obtain more robust data. In this case, the actual median CV for the low range was 22.47% (14.9%-31% interquartile range). Again, most of the allergens depicted consistent results in terms of sensitization outcome (positive vs negative results), except for Alt a 6 (CV: 108%, median, range: 0.32, 0.10-1.89 kU_A/L), Ara h 15 (CV: 183%, median, range: 0, 0-0.71 kU_A/L), and Der p 5 (CV: 93%, median, range: 0.39, 0-0.55 kU_A/L). Two other allergens having positive and negative results in different measurements presented moderate variability and data close to the cutoff values: Phl p 5.0101 (CV: 36%, median, range: 0.27, 0.26-0.53 kU_A/L) and Phl p 6 (CV: 39%, median, range: 0.36, 0.28-0.69 kU_A/L). Considering the consistency of data between different samples, in general, high ICC was observed for interassay determinations (Table 5).

Interbatch and interlaboratory variabilities were calculated by analyzing 2 measurements under the following different conditions: different batch and different laboratory, respectively. ICC was calculated for interbatch and interlaboratory analysis (Table 5), including the pool, along with 9 samples. Regarding consistency of data, from 440 determinations evaluated in interbatch analysis (44 allergens in 10 sera), only 17 (3.9%) depicted differences that led to a different sensitization diagnostic (positive vs negative sIgE). Moreover, from 440 determinations evaluated in the interlaboratory analysis, only 11 (2.5%) depicted differences that led to a different sensitization diagnosis.

Linearity analysis was performed for those allergens having measurements equal or more than 3 kU_A/L, to ensure dilutions fell within the positive value range of the technique. Using this criterion, we included 59 measurements in this analysis, covering 28 of our 44 studied allergens. Linearity of sIgE by ALEX2 platform has been reported,[15] with an upper limit of detection described at 50 kU_A/L. We observed that, for allergens with high sIgE concentrations (>30 kU_A/L), there was a decrease of the slope and the regression coefficient (R^2) values (Fig 1), suggesting poor linearity in the upper range. For concentrations lower than 30 kU_A/L and higher than 0.3 kU_A/L,

Table 4
Intra-Assay and Interassay CV Obtained With the Pool of Sera

Median sIgE range (kU_A/L)		Median intra-assay CV, % (IQR)		Median interassay CV, % (IQR)
<0.30	(N = 12)		(N = 11)	
≥0.30 to <1	(N = 4)	27.6 (23.7-30.9)	(N = 5)	39.1 (14.4-93.4)
≥1 to <10	(N = 16)	6.5 (5.1-8.2)	(N = 15)	11 (8.5-14.9)
≥10	(N = 12)	4.5 (3.1-7.1)	(N = 13)	8.1 (7.2-14.4)

Abbreviations: CV, coefficient of variability; IQR, interquartile range; sIgE, specific immunoglobulin E.
NOTE. Allergens were classified in ranges according to their median sIgE. Median CV and IQRs were calculated for each range (number of allergens used reported). Only allergens with a median value ≥ 0.3 kU_A/L were considered.

Table 5
Interassay (N = 5 Determinations), Interbatch (N = 2 Determinations), and Interlaboratory (N = 2 Determinations) Intraclass Coefficient of Variability Obtained for the 44 Allergens Evaluated

Allergen		Interassay ICC	Interbatch ICC	Interlaboratory ICC
Cypress pollen	Cup a 1	0.996	0.998	1
Grass pollen (*Phleum pratense*)	Phl p 1	0.995	0.965	0.99
	Phl p 2	0.993	0.996	0.999
	Phl p 5.0101	0.973	0.977	0.992
	Phl p 6	0.972	0.983	0.983
	Phl p 7	0.998	0.878	0.999
	Phl p 12	0.986	0.763	0.995
Olive pollen	Ole e 1	0.996	0.998	0.981
	Ole e 7 RUO	0.773[a]	—[a]	−0.179[a]
	Ole e 9	0.988	0.963	0.998
Alternaria alternata	Alt a 1	0.998	1	0.99
	Alt a 6	0.915	**0.684**	0.841
Dermatophagoides pteronyssinus	Der p 1	0.998	0.997	1
	Der p 2	0.999	0.999	1
	Der p 5	0.999	0.998	1
	Der p 7	0.997	**0.680**	1
	Der p 10	**0.6**	−0.385[a]	−0.274[a]
	Der p 11	**0.678**	−0.372[a]	−0.78[a]
	Der p 20	0.995	0.987	0.998
	Der p 21	0.998	1	1
	Der p 23	0.999	0.998	1
Apple	Mal d 1	0.998	0.987	0.979
	Mal d 2	0.884	−**0.88**	−0.399[a]
	Mal d 3	0.989	0.948	0.991
Peach	Pru p 3	0.972	−**0.390**	0.998
	Pru p 7 RUO	0.233[a]	0.737[a]	0[a]
Hazelnut	Cor a 1.0401	0.981	0.966	0.969
	Cor a 8	0.993	0.996	0.998
	Cor a 9	0.989	0.958	0.994
	Cor a 11	0.989	0.999	0.999
	Cor a 12 RUO	0.047[a]	−0.175[a]	0[a]
	Cor a 14	0.999	0.965	0.997
Peanut	Ara h 1	0.999	0.975	0.99
	Ara h 2	0.999	0.989	0.99
	Ara h 3	0.989	0.981	0.808
	Ara h 6	0.998	0.988	0.999
	Ara h 8	0.97	0.932	0.861
	Ara h 9	0.989	0.754	0.996
	Ara h 15	0.848	**0.446**	0.61[a]
Walnut	Jug r 1	0.999	0.996	0.999
	Jug r 2	0.999	0.995	0.969
	Jug r 3	0.989	0.976	0.987
	Jug r 4	0.993	0.998	0.998
	Jug r 6	0.998	0.990	0.995

Abbreviations: ICC, intraclass coefficient correlation; RUO, research use only; sIgE, specific immunoglobulin E.
NOTE. ICCs below 0.7 for allergens having positive mean sIgE are marked in bold.
[a]Allergen depicted negative mean sIgE values in all the samples of the analysis (<0.30 kU_A/L).

most of the measurements depicted good linearity. Within this concentration range, only 6 linear regressions of 48 had very poor metrics (Fig 1), including Alt a 6 (analyzed in 1 sample), Ara h 3 (in 2 of 2 analyzed samples), Cup a 1 (in 1 sample of 4 analyzed samples), Mal d 1 (in 1 sample of 2 analyzed samples), and Pru p 3 (in 1 sample of 4 analyzed samples). To note, Alt a 6 had the worst linearity data, consistently depicting a deficient performance in our technical analysis.

Discussion

Allergy diagnosis has largely benefited from multiplex allergen platforms based on molecular components. Ever since the ISAC was introduced 20 years ago,[16] other techniques have been developed, each one with its own set of allergens and technical features.[17,18] Our study sought to evaluate the diagnostic performance of 1 of the newest platforms, ALEX version 2 (ALEX2), comparing it with the reference standard in sIgE quantification, ImmunoCAP, and the most widely used multiplex array, ImmunoCAP ISAC112, in a properly characterized sample.

In general, the ALEX2 platform has sound capacity for the detection of patients with allergy to some aeroallergens, certain fruits, and nuts, proving to be a reliable tool for molecular diagnosis. ALEX2 diagnostic performance is quite similar to that of the previous existing platforms, ISAC112 and ImmunoCAP, for most of the studied allergens. Nevertheless, exceptions to the above-mentioned statement are evident in the capacity for detection of patients with allergy to olive pollen and those with allergy to walnut based on Jug r 3 performance. When interpreting data pertaining to olive pollen component detection, a low sensitivity was found in general. Sensitivity is much improved when adding to the analysis of Fra e 1, a molecular component of the ash tree pollen (an Oleacea tree), which has high identity with Ole e 1.[19] Fra e 1 seems to expose relevant olive pollen epitopes better than the Ole e 1 molecule in the ALEX2 chip. Until the Ole e 1 molecule is improved, it may be wise to consider Fra e 1 in olive pollen diagnosis when interpreting ALEX2 results in cases having a clinical profile compatible with olive pollen allergy. Moreover, although ALEX2 has extended the spectrum of walnut allergens represented in the chip, and Jug r 1 and Jug r 2 seem to be diagnosed similarly to those in ISAC112, detection of patients with allergy is strengthened by Jug r 3 because ours is predominantly sensitized to the walnut lipid transfer protein.

ALEX2 was found to have improved diagnostic capacity for *Alternaria* and apple allergies compared with ISAC112, owing to the better performance of Alt a 1 and the inclusion of Mal d 3, respectively. It is important to consider that in terms of component-resolved diagnosis, extensively wide panels of allergens allow the inclusion of clinically relevant allergens, such as Der p 23,[9,20] absent in ISAC112. In fact, 15% of patients with allergy to *D pteronyssinus* had sensitization to this component without sensitization to the classic major allergens, being misdiagnosed by ISAC112 (analyzing Der p 1, Derp 2, Derp 10). The addition of whole extracts to the sample component panels in these new platforms, also reported in other platforms,[5] seems to increase sensitivity for certain allergenic sources as well. Even though ALEX2 includes whole extract detection for some allergens, these extracts belong to biological sources other than those considered in our analysis.

The diagnostic capacity of ALEX2 seems to be quite similar to that of ISAC112 in terms of qualitative data. Furthermore, ALEX2 is a quantitative technique having good correlation with sIgE by ImmunoCAP. In terms of equivalence of sIgE, different levels of sIgE have been detected using ALEX2 and ImmunoCAP. Because ImmunoCAP was found to have a good agreement between the use of ng/mL and kU_A/L units as a measurement of sIgE by external laboratories as well,[21] we believe that a similar external validation should be performed for ALEX2 and for different allergens and concentrations. We noted that the quantification in the upper scale of the dynamic range (more than 30 kU_A/L) depicted poor linearity, suggesting the actual dynamic range is lower than 50 kU_A/L. This has been recently reported by some authors who suggest that the dynamic range is between 0.30 and 30 to 40 kU_A/L, in line with our findings.[8,11] Nevertheless, we consider that low linearity in these value ranges is not of relevance, as values this high are not frequent, and linear quantification at this point is of low clinical consequence.[22,23]

Total IgE detection is comparable between ALEX2 and ImmunoCAP. Given the importance of considering total IgE when interpreting sIgE results, this may represent a cost-effective improvement over ISAC112, which requires a separate ImmunoCAP singleplex assay to measure total IgE.[24]

When conducting our technical analysis, we observed that the technique exhibits good reproducibility and repeatability performance in our hands. As found with intra- and interassay variability data, the technique generally provides robust sIgE values. Higher variability was observed in lower range values, because CV is highly influenced by mean value, something that has been also noted for ISAC112.[25] Nevertheless, CV was quite high for some allergens, such as Alt a 6. The technique also proved to be robust in the interbatch and interlaboratory assessment, in which most of the allergens depicted excellent results and with a very low percentage of sensitization data disagreements. As a limitation, we acknowledge that our technical analysis is not based on all the allergens available in the array, focusing only on a set of allergens of interest. Thus, overall results could slightly vary.

As previously commented by other authors,[7] the ALEX2 microarray contains a broader panel of allergens to evaluate sensitizations using a top-down approach, offering a global test at a lower cost per patient than the ISAC112. In addition, ALEX2 can be automatized, thus reducing the effect and need of human handling and shortening the time taken to run each test when compared with ISAC112. Therefore, ALEX2 emerges as a promising tool, especially in polysensitized patients. After validation of all the allergens present in ALEX2, cost-effectiveness analysis studies comparing different available techniques should be performed.

In conclusion, according to our study results, ALEX2 is a microarray having adequate diagnostic capacity, comparable with that of the ISAC112 multiplex and ImmunoCAP singleplex, revealing sIgE ImmunoCAP-correlated quantitative results up to 30 kU_A/L. Furthermore, repeatability and reproducibility of the ALEX2 platform have been found. The spectrum of application of our results is limited to patients with Mediterranean sensitization profiles, so further evaluations are recommended in other regions for patients with varying allergenic exposures.

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Appendix. Supplementary materials

eMethods

Patients

We included 169 subjects: 153 patients with allergy and 16 controls without atopy. The selected patients with allergy to aeroallergen met the following criteria: (1) a history of respiratory symptoms (allergic rhinoconjunctivitis or asthma) during the corresponding pollination season, dust mite season, or Alternaria spore-spread period during the 2 years before inclusion; (2) a positive skin prick test result to the culprit allergen; and (3) geographic stability, as defined by having lived in the same geographic area for the past 5 years. Pollination and *Alternaria* spore-spread calendars in each recruitment zone were defined according to the Aerobiology Committee of the Spanish Society of Allergology and Clinical Immunology. For patients with allergy to *Alternaria*, a positive sIgE level to *Alternaria alternata* was considered an additional inclusion criterion. Exclusion criteria for patients with pollen and *Alternaria* allergy were defined as skin sensitization to other pollen or *Alternaria* with overlapping pollination/spore-spread periods as the causative allergen. In patients with dust mite allergy, skin sensitization to pets was excluded.

The selected patients with food allergy met the following inclusion criteria: (1) a clear history of IgE-mediated symptoms—defined as oral allergy syndrome, contact urticaria, nonanaphylactic systemic symptoms (urticaria, angioedema, respiratory, or gastrointestinal symptoms), or anaphylaxis—after consumption of the culprit food; (2) a positive skin prick test result to the corresponding allergen; and (3) positive sIgE levels ($\geq$0.35 kU_A/L, ImmunoCAP, Thermo Fisher Scientific) to the whole extract of the causative allergen.

The control group for each allergen was formed by patients without atopy (N = 16) and patients with atopy (used as patients with allergy for other allergens) depending on the studied allergen.

Skin prick tests were performed in all participants with commercial extracts of cypress pollen, grass pollen mix, olive pollen, *Dermatophagoides pteronyssinus*, apple, peach (30 mg/mL of Pru p 3), walnut, hazelnut, and peanut (ALK, Hørsholm, Denmark), and *A alternata* (Diater, Madrid, Spain). Sodium chloride (0.9%) and histamine hydrochloride (10 mg/mL, ALK) served as negative and positive controls, respectively. Wheals of 3 mm in diameter were considered positive, as recommended by the European Academy of Allergy and Clinical Immunology guidelines.

Sera for Validation Analysis

For technical validation, sera from patients attending to the Allergy Department of Clínica Universidad de Navarra having polysensitization when tested with ALEX version 1 were reviewed. There were 9 sera revealing detectable amounts of sIgE against the 44 allergen components from the 9 evaluated allergen sources which were selected. In addition, 6 sera (5 from the 9 previously selected plus an extra new one) were pooled together to obtain a sample with a broad variety of sIgE. This pool was handled as an extra sample.

Multiplex-Specific Immunoglobulin E Assay

Specific IgE to allergens of the *Cupressus arizonica* (Cup a 1), *Phleum pratense* (Phl p 1, Phl p 2, Phl p 5.0101, Phl p 6, Phl p 7, Phl p 12), *Olea europea* (Ole e 1, Ole e 7, and Ole e 9), *D pteronissinuss* (Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, Der p 21, Der p 23), and *A alternata* (Alt a 1, Alt a 6), apple (Mal d 1, Mal d 2, Mal d 3), peach (Pru p 3, Pru p 7), walnut (Jug r 1, Jug r 2, Jug r 3, Jug r 4, Jug r 6), hazelnut (Cor a 1.0401, Cor a 8, Cor a 9, Cor a 11, Cor a 12, Cor a 14), and peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9, Ara h 15) was measured in all patients by means of ALEX version 2 (ALEX2) (MacroArray Diagnostics). Allergens Ole e 7, Pru p 7, and Cor a 12 are considered for research use only (RUO) by the manufacturer. Patients' sera were incubated for 2 hours in the chip, containing the different extracts and component allergens spotted onto a nitrocellulose membrane. Afterward, the chips are extensively washed, and a pretitered dilution of an antihuman IgE labeled with alkaline phosphatase is added and incubated for 30 minutes. Following another cycle of extensive washing, the enzyme substrate is added and the presence of IgE is developed by colorimetry in the nitrocellulose paper. The membranes are dried and scanned.

Specific IgE to allergens of *C arizonica* (Cup a 1), the grass *P pratense* (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12), *O europea* (Ole e 1, Ole e 7, and Ole e 9), *D pteronyssinus* (Der p 1, Der p 2, Der p 10), and *A alternata* (Alt a 1, Alt a 6), apple (Mal d 1), peach (Pru p 1, Pru p 3, Prup 4), walnut (Jug r 1, Jug r 2, Jug r 3), hazelnut (Cor a 1.0401, Cor a 8, Cor a 9), and peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9) was measured in all patients by means of ImmunoCAP ISAC 112 (ThermoFisher). sIgE against the described allergens was evaluated following manufacturer's instructions. Briefly, each microarray is incubated with a serum to label sIgE to each protein, and subsequently it is incubated with a human anti-IgE detection antibody fluorescently labeled. Finally, the fluorescence intensity of each microarray is measured by the scanner (LuxScan 10K/A, CapitalBio, Beijing, People's Republic of China), and images are digitalized by the Microarray Image Analyzer software (ThermoFisher Scientific). The software allows transforming the fluorescence intensity in numerical data according to the calibration curve built with a calibrator sample included in each assay.

eTable 1
List of Whole Extracts (Bold) and Components Analyzed in Each Technique for the Selected Biological Sources

Biological sources	ALEX2	ISAC112	ImmunoCAP
Cupressus arizonica			**t222**
	Cup a 1	Cup a 1	Cup a 1
Phleum pratense			**g6**
	Phl p 1	Phl p 1	Phl p 1
	Phl p 2	Phl p 2	
	Phl p 3		
		Phl p 4	
	Phl p 5.0101	Phl p 5	
	Phl p 6	Phl p 6	
	Phl p 7	Phl p 7	
		Phl p 11	
	Phl p 12	Phl p 12	
Olea europea			**t9**
	Ole e 1	Ole e 1	Ole e 1
	Ole e 7 (RUO)	Ole e 7	
	Ole e 9	Ole e 9	
Dermatophagoides pteronyssinus			**d1**
	Der p 1	Der p 1	Der p 1
	Der p 2	Der p 2	Der p 2
	Der p 5		
	Der p 7		
	Der p 10	Der p 10	
	Der p 11		
	Der p 20		
	Der p 21		
	Der p 23		Der p 23
Alternaria alternata			**m6**
	Alt a 1	Alt a 1	Alt a 1
	Alt a 6	Alt a 6	
Apple			**f49**
	Mal d 1	Mal d 1	
	Mal d 2		
	Mal d 3		Mal d 3
Peach			**f95**
		Pru p 1	
	Pru p 3	Pru p 3	Pru p 3
		Pru p 4	
	Pru p 7 (RUO)		
Walnut			**f256**
	Jug r 1	Jug r 1	
	Jug r 2	Jug r 2	
	Jug r 3	Jug r 3	Jug r 3
	Jug r 4		
	Jug r 6		
Hazelnut			**f17**
	Cor a 1.0401	Cor a 1.0401	
	Cor a 8	Cor a 8	Cor a 8
	Cor a 9	Cor a 9	
	Cor a 11		
	Cor a 12 (RUO)		
	Cor a 14		
Peanut			**f15**
	Ara h 1	Ara h 1	
	Ara h 2	Ara h 2	
	Ara h 3	Ara h 3	
	Ara h 6	Ara h 6	
	Ara h 8	Ara h 8	
	Ara h 9	Ara h 9	Ara h 9
	Ara h 15		

Abbreviations: ALEX2, Allergy Explorer version 2; ISAC112, Immuno Solid-phase Allergen Chip 112; RUO, research use only.

eTable 2A
Demographic and Clinical Data of Patients With Allergy to the Evaluated Respiratory Allergens and the Corresponding Control Group

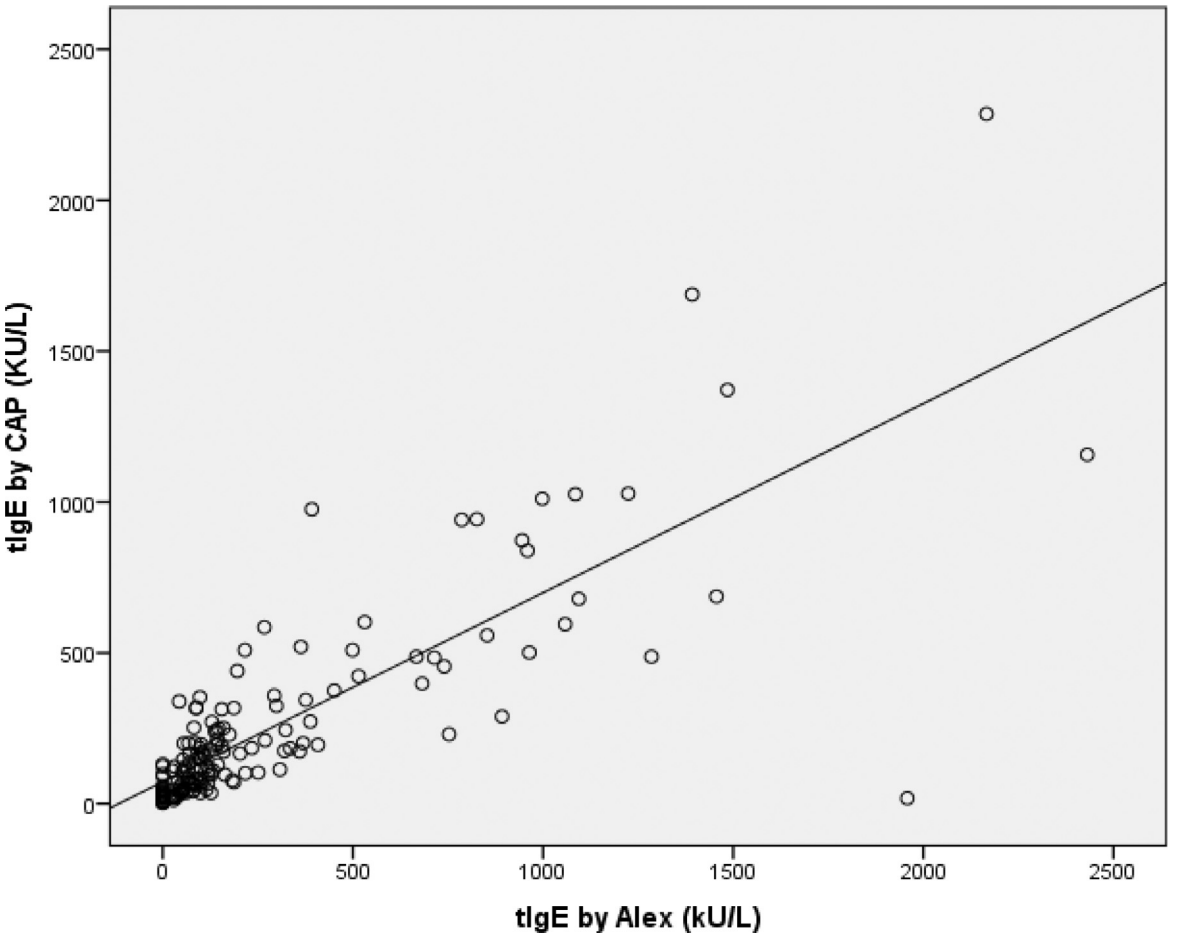
Biological sources	Cypress pollen		Grass pollen		Olive pollen		*Dermatophagoides pteronyssinus*		*Alternaria alternata*	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
N	28	26	29	27	27	31	20	22	18	22
Age, y	29	44	31	35	32	44	31.5	40.5	40.5	40.5
Median (IQR)	(23-35.5)	(31-50.5)	(25-38)	(25-49)	(25-36)	(31-53)	(26-40)	(29-54)	(36-42)	(29-54)
Sex, male, %	17.8	26.9	34.5	14.8	28.6	16.1	55.0	22.7	22.2	27.2
Whole-extract sIgE ImmunoCAP,	2.83	0.01	5.57	0.02	2.97	0	12.45	0.01	7.05	0
kU_A/L Median (IQR)	(0.76-6.37)	(0-0.02)	(2.22-27.25)	(0-0.05)	(0.62-16.1)	(0-0.06)	(3.11-25.35)	(0-0.02)	(3-9.86)	(0-0)
Rhinitis, %	100	42.9	93.1	50	92.6	48.3	100	9.1	100	22.7
Asthma, %	42.9	4.7	31.0	8.3	22	10	50	0	77.8	4.7
Conjunctivitis, %	85.7	28.6	82.8	20.8	74.1	31.0	89.5	4.5	100	13.6

Abbreviations: IQR, interquartile range; sIgE, specific immunoglobulin E.

eTable 2B
Demographic and Clinical Data of Patients With Allergy to the Evaluated Food Allergens and the Corresponding Control Group

Biological sources	Apple		Peach		Walnut		Hazelnut		Peanut	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
N	15	23	34	23	28	23	20	23	19	23
Age, y	29	45	30	45	22	45	28	45	29	45
Median (IQR)	(21-38)	(32-54)	(24-38)	(32-54)	(16-33)	(32-54)	(22-37)	(32-54)	(19-39)	(21-54)
Sex, male, %	40	13	32.5	13	35.7	13	30	13	36.8	13
Whole-extract sIgE ImmunoCAP,	2.0	0	4.94	0	1.73	0	1.52	0	1.63	0
kU_A/L Median (IQR)	(1.28-3.93)	(0-0.02)	(1.78-9.69)	(0-0)	(1.12-3.38)	(0-0.01)	(1.01-4.07)	(0-0)	(0.59-6.22)	(0-0.01)
Oral allergy, %	26.7	0	41.2	0	64.3	0	45	0	21.05	0
Contact urticaria, %	0	0	0	0	0	0	0	0	5.3	0
Nonanaphylactic systemic symptoms, %	40	0	35.3	0	28.6	0	35	0	52.6	0
Anaphylaxis, %	33.3	0	23.5	0	7.1	0	20	0	21.05	0

Abbreviations: IQR, interquartile range; sIgE, specific immunoglobulin E.



eFigure 1. Correlation between total IgE (kU/L) measured by ALEX2 and ImmunoCAP. ALEX2, Allergy Explorer version 2; tIgE, total immunoglobulin E.