Comparison of Molecular Multiplex and Singleplex Analysis of IgE to Grass Pollen Allergens in Untreated German Grass Pollen–Allergic Patients

Ahlgrim C¹, Gutermuth J², Önell A³, Borres MP³,⁴, Schäffner I⁵, Darsow U⁶,⁷, Pfab F⁶,⁷, Brockow K⁶,⁷, Ring J⁶, Behrendt H⁷, Jakob T⁸, Huss-Marp J⁵,⁸

¹University Freiburg Medical Center, Department of Exercise Medicine and Sport, University of Freiburg, Freiburg, Germany
²Department of Dermatology, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium
³Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden
⁴Department of Women's and Children’s Health, Uppsala University, Uppsala, Sweden
⁵Thermo Fisher Scientific, Phadia GmbH, Freiburg, Germany
⁶Department of Dermatology and Allergy, Technische Universität München, Munich, Germany
⁷ZAUM-Center for Allergy and Environment, Helmholtz Center Munich, Technische Universität München, Munich, Germany
⁸Allergy Research Group, Department of Dermatology, Medical Center, University of Freiburg, Freiburg, Germany

Abstract

Background: The ImmunoCAP ISAC 112 platform is the only commercially available molecular allergy IgE multiplex test. Data on the comparison of this rather novel test with the molecular singleplex ImmunoCAP IgE platform are lacking.

Objective: To compare the multiplex ISAC 112 platform and the singleplex ImmunoCAP platform in regard to IgE to grass pollen allergens in untreated grass pollen–allergic patients in Germany.

Methods: Serum samples from 101 adults with grass pollen allergy were analyzed for specific IgE (sIgE) to 8 allergenic molecules from timothy grass pollen and to the 112 allergenic molecules included in the ISAC panel. The results for the multiplex and singleplex tests were subsequently analyzed statistically.

Results: Comparison of sIgE to grass pollen allergens detected by ISAC 112 and the singleplex ImmunoCAP assay revealed the following correlation coefficients: 0.88 (rPhl p 1), 0.96 (rPhl p 2), 0.70 (nPhl p 4), 0.94 (rPhl p 5b), 0.92 (rPhl p 6), 0.85 (rPhl p 11), and 0.78 (rPhl p12).

Conclusion: Molecular testing with ISAC 112 correlates well with the ImmunoCAP platform for respective molecular timothy grass pollen allergens.

Key words: Molecular allergy. Multiplex. ImmunoCAP. ISAC. Component-resolved diagnosis.
Introduction

The recently published WAO-ARIA-GA2LEN consensus document [1] provides clinicians with a practical guide regarding the indications, determination, and interpretation of molecular allergy (MA) diagnostics. More than 130 allergenic molecules are currently available for in vitro specific IgE (sIgE) testing, which can be performed on singleplex or multiplex measurement platforms. In this consensus document MA diagnostics is suggested as a third-line approach in patients with an inconclusive diagnostic outcome based on clinical history and extract-based IgE allergen tests (in vitro sIgE or skin prick testing). The recommendation to use MA diagnostics to complement conventional allergy diagnostic testing has produced a now-common clinical situation where extract-based IgE test results are linked to MA test results (eg, birch pollen to Bet v 1). This approach is complicated because IgE results cannot be easily compared across assays from different manufacturers [2,3] or across different analytical platforms, such as multiplex vs singleplex systems. A number of studies addressing this have compared the Immuno-Solid phase Allergen Chip (ImmunoCAP ISAC), the only MA multiplex platform available on the market to date, with ImmunoCAP singleplex tests (both Thermo Fisher Scientific) [4-12]. However, these studies investigated previous versions of the ISAC assay, which contained an allergen panel of 103 allergens. The current ISAC (ImmunoCAP ISAC 112) was launched in 2011 with an extended allergen panel and improved performance characteristics. While a recent study reported on the accuracy, precision, repeatability, and reproducibility of this updated platform [13], no data have yet been published on how it compares to the ImmunoCAP sIgE singleplex test.

The aim of this study was to compare the current version of the ISAC multiplex IgE assay (from here on referred to as ISAC 112) and ImmunoCAP singleplex IgE tests (ImmunoCAP) in regard to IgE to grass pollen allergens in untreated patients with grass pollen allergy to provide practitioners with information on how to best interpret sIgE results as a basis for appropriate clinical conclusions.

Methods

Patients and Study Design

Sera from 101 adults (median age, 27 years; 58.4% females) with allergic rhinoconjunctivitis and diagnosed allergy to timothy grass pollen from Munich, Germany were analyzed. Inclusion criteria were a serum level of IgE against timothy grass pollen (Phleum pratense) of 0.35 kU/L or higher and a positive history of grass pollen allergy. Pregnant patients and patients with concomitant disease were excluded. None of the patients were being treated with allergy-related systemic medication and were not undergoing and had never undergone specific immunotherapy. Further details on the data set of this study population have been previously published [14,15].

Informed consent was obtained from all individuals before their participation in the study. The study protocol was approved by the local ethics committee prior to the start of the study and is in line with the principles of the latest revision of the Declaration of Helsinki.

Comparison of ISAC 112 and ImmunoCAP

Serum samples were analyzed for sIgE using ImmunoCAP for allergenic molecules from timothy grass pollen and ISAC 112.

The ImmunoCAP singleplex tests were performed according to the manufacturer’s instructions. The quantitation range for sIgE is 0.1 to 100 kU/L and the mean within- and between-assay coefficient of variation (CV) is 4% for values between 0.35 and 1.5 kU/L according to the manufacturer [http://www.dfu.phadia.com/]. For the ISAC 112 test, a solid-phase immunoassay that allows simultaneous sIgE detection against 112 allergenic molecules from 51 different allergenic sources was performed as described elsewhere [13]. The measuring range is 0.3 to 100 ISU-E and the limit of detection is less than 0.3 ISU-E for all allergenic molecules. The within- and between CV per component is 14% and 8% respectively for values ranging from 0.3 to 1.0 ISU-E [http://www.dfu.phadia.com/].

For MA testing, ImmunoCAP was used to test for IgE against 8 single allergenic molecules of Phl p pollen, namely the recombinant rPhl p 1 (g205), rPhl p 2 (g206), rPhl p 5b (g215), rPhl p 6 (g209), rPhl p 7 (g210), rPhl p 11 (g211) and rPhl p 12 (g212), and the native nPhl p 4 (g208).

ISAC 112 was used to analyze all patient samples, yielding a total of 11 312 test results (101 samples x 112 allergenic molecules), all based on triplicate measurements due to the setup of the allergen chip.

Subsequently, the ImmunoCAP results for the Phl p allergenic molecules rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5b, rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 2 were compared with the corresponding allergens on the ISAC 112 platform.

Resultados: La comparación de los valores de IgE específica frente a los alérgenos de pólenes de gramíneas hallados en los sistemas ISAC e ImmunoCAP mostraron los siguientes coeficientes de correlación: 0.88 (rPhl p 1), 0.96 (rPhl p 2), 0.70 (nPhl p 4), 0.94 (rPhl p 5b), 0.92 (rPhl p 6), 0.85 (rPhl p 11) y 0.78 (rPhl p 12).

Conclusiones: El diagnóstico molecular con el Sistema ISAC guarda buena correlación con los resultados del ImmunoCAP para los alérgenos de hierba timotea presentes en ambas plataformas.

Palabras clave: Alergia molecular. Multiplex. ImmunoCAP. ISAC. Diagnóstico por componentes.
Statistical Analysis

Statistical analysis was performed to compare ISAC 112 and ImmunoCAP sIgE results for both allergen molecules and extracts. The Spearman rank order correlation coefficient (ρ) for the corresponding IgE values was thus calculated for both methods (Figure).

Results

IgE Test Results

At the molecular level, the most common \( \text{Phl} p \) allergens responsible for sensitizations were \( \text{rPhl} p 1 \) and \( n\text{Phl} p 4 \) (both 92%), followed by \( \text{rPhl} p 5b \) (81%). The lowest prevalence was found for \( \text{rPhl} p 7 \), which showed sensitizations in just 2 of 101 patients. Both had positive results with the ISAC 112 and ImmunoCAP systems; 3 additional patients showed sensitization to \( \text{rPhl} p 7 \) in just 1 of the systems. Individual sensitization levels for the \( \text{Phl} p \) allergen molecules are shown in the Table.

Sensitization to the cross-reactive carbohydrate (CCD) marker MUXF3 was detected in 19.8% of \( n\text{Phl} p 4 \)-positive patients, but with limited correlation (\( ρ=0.345 \)). Since MUXF3 is not always the best marker for CCD reactivity, the CCD-carrying \( n\text{Jug} r 2 \) was also analyzed in this context (17.4%, \( ρ=0.241 \)). The results imply that the high rate of \( n\text{Phl} p 4 \) sensitization in our patients was mostly species-specific and not due to cross reactivity to cross-reactive carbohydrates, as indicated by the CCD markers MUXF3 and \( n\text{Jug} r 2 \).

ISAC 112 tests performed with sera from 101 patients produced 112 separate results for each individual and are thus too extensive to be shown here in detail. Since the focus of this publication is to compare grass pollen allergen results for the different test platforms, we show only ISAC 112 data in relation to the 8 \( \text{Phl} p \) allergen molecules tested using the singleplex ImmunoCAP system (Figure). All values below the detection limits of 0.3 ISU for ISAC and 0.1 kU/L for ImmunoCAP are expressed as 0.01 ISU or 0.01 kU/L, respectively, in order to show them in the logarithmic graphs in the Figure. For the calculation, all samples below detection limits were set to zero.

Comparison of ISAC 112 and ImmunoCAP

Results for the 8 \( \text{Phl} p \) allergenic molecules were compared between the multiplex ISAC 112 and the singleplex ImmunoCAP platforms. The calculations revealed the following correlation coefficients: 0.88 (\( \text{rPhl} p 1 \) & 0.96 (\( \text{rPhl} p 2 \), 0.70 (\( n\text{Phl} p 4 \), 0.94 (\( \text{rPhl} p 5b \), 0.92 (\( \text{rPhl} p 6 \), 0.85 (\( \text{rPhl} p 11 \)), and 0.78 (\( \text{rPhl} p 12 \)). All correlations were statistically significant. All \( P \) values were less than .0001 except for \( \text{rPhl} p 7 \) (\( P=.0078 \)). The correlations are displayed in Figure A-H. As there were just 2 sensitizations against \( \text{rPhl} p 7 \) in our patient series, these results were excluded from the analysis.

Discussion

This is the first study to compare the current ISAC 112 multiplex platform with the ImmunoCAP singleplex platform in regard to both extract-based and molecular in vitro sIgE tests.

The correlation analysis revealed that the 2 systems were closely correlated for the corresponding molecular allergens, although ISAC 112 produced slightly higher values at higher IgE levels. However, at low levels (<1-2 kU/L), it missed slightly more samples, indicating sample dependency. Finally, our data indicate that ISAC 112 and ImmunoCAP results are not interchangeable due to the different technologies used. Even though close correlations were found, these have to be considered at the individual allergen level and do not permit the definition of a general factor for transferring test results from one method to the other.

MA diagnostics is being increasingly used in routine clinical practice worldwide, providing an enhanced diagnostic depth that complements conventional extract-based sIgE testing. The added diagnostic value of MA is favoring its routine clinical use, particularly in the context of food allergy or prior to specific immunotherapy in polysensitized individuals with pollinosis [9,16-18]. Considering its increasing use, there is a growing need for information on proper application techniques and correct interpretation of MA results. Regarding the sequence of diagnostic steps, the authors of the consensus document generally consider MA to be a third-line approach to be used in the case of inconclusive first- and second-line investigations, which generally provide sufficient

Table. Sensitization Characteristics of Patients With Allergic Rhinoconjunctivitis (n=101)

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. (%)</th>
<th>Age, y</th>
<th>Serum specific IgE, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42 (41.6)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Female</td>
<td>59 (58.4)</td>
<td>30.2 (10.0)</td>
<td>27 (18-64)</td>
</tr>
<tr>
<td>Age, y</td>
<td>27 (20-63)</td>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td>Serum specific IgE, kU/L</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td>Timothy pollen*</td>
<td>20.0 (25.5)</td>
<td>10.5 (0.4-127)</td>
<td></td>
</tr>
<tr>
<td>Timothy pollen\†</td>
<td>18.3 (21.1)</td>
<td>9.81 (0.3-157)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 1 )</td>
<td>10.5 (14.8)</td>
<td>5.1 (0-79)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 2 )</td>
<td>1.9 (4.5)</td>
<td>0.5 (0-38)</td>
<td></td>
</tr>
<tr>
<td>( n\text{Phl} p 4 )</td>
<td>4.1 (5.2)</td>
<td>2.1 (0.1-31)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 5b )</td>
<td>9.4 (15.9)</td>
<td>3.9 (0-98)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 6 )</td>
<td>2.3 (3.9)</td>
<td>0.8 (0-24)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 7 )</td>
<td>0.16 (1.5)</td>
<td>0 (0-15)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 11 )</td>
<td>1.2 (2.6)</td>
<td>0.1 (0.2-16)</td>
<td></td>
</tr>
<tr>
<td>( r\text{Phl} p 12 )</td>
<td>0.2 (0.4)</td>
<td>0.1 (0.2-4)</td>
<td></td>
</tr>
<tr>
<td>Serum total IgE, IU/mL</td>
<td>237 (298)</td>
<td>116 (0-1664)</td>
<td></td>
</tr>
<tr>
<td>Serum total IgE, IU/mL</td>
<td>247 (326)</td>
<td>115 (2-1750)</td>
<td></td>
</tr>
</tbody>
</table>

\*During grass pollen season.
\†Outside grass pollen season.
information in the majority of patients. For experienced users, MA diagnostics may be used at an earlier stage and could even be included in second-line allergy testing. However, ISAC testing is generally reserved for challenging cases and used at a later diagnostic stage [1]. ISAC testing is therefore usually performed as a last step and is thus frequently compared with previous singleplex MA tests.

On comparing ISAC 112 and ImmunoCAP results for *Phl* pollen allergens (excluding *Phl p 7* due to too few observations), we observed a significant correlation, and also saw that ImmunoCAP offered higher sensitivity for *Phl p 1*, *Phl p 2*, *Phl p 5*, *Phl p 6*, *Phl p 11*, and *Phl p 12*. These findings are in line with previous investigations [7] and are also discussed in the WAO-ARIA-GA2LEN consensus document [1]. The reason for the higher sensitivity of the singleplex ImmunoCAP test at the molecular level is the technology used, as it incorporates a nitrocellulose sponge with high binding capacity able to bind allergens in the microgram range. This means that the bound allergenic molecule is available in excess in the ImmunoCAP system for the detection of the corresponding sIgE in the patient serum. By contrast, because of its microarray design, allergens are applied to the ISAC 112 chip in picogram quantities. In short, 100 pg allergen are immobilized on a single spot of the chip (spot size 200 µm). This quantity is 10 000 times less than that used in the ImmunoCAP system. An additional factor that could influence sensitivity is allergen binding to the test surface, which differs between the 2 technologies. Finally, ISAC 112 is classified as a semi-quantitative test due to its calibration system, unlike ImmunoCAP, which is calibrated to the WHO standard for total IgE (WHO IRP 75/502). Based on these considerations, it is clear that in regard to grass pollen allergens and the context of the experimental work outlined above, ISAC 112 is less sensitive than ImmunoCAP. In addition, however, while the results of the 2 assays are similar, they are not interchangeable, as indicated in the WAO-ARIA-GA2LEN consensus document [1]. Our data show that for some allergens, notably *Phl p 4*, *Phl p 11*, and *Phl p 12*, a significant number of sera were clearly positive with one system but...
negative with the other. This also occurred with *Phl p 7*, but to a lesser degree. This effect appears to be allergen-dependent and could be due to underlying technological differences between the 2 methods (e.g. allergen binding to test surface, coverage of epitopes, etc.).

Furthermore, our study has an important limitation in that our findings apply only to patients who have never undergone specific immunotherapy; in patients who have undergone this treatment, an increased sIgG response could have a different impact on IgE tests with ISAC 112 and ImmunoCAP, as these differ substantially in terms of the quantity of allergen fixed to the 2 matrices, as indicated above.

In conclusion, the multiplex ISAC 112 platform correlated well with the singleplex ImmunoCAP platform for timothy grass pollen allergens, which is important when performing comparisons in the course of the diagnostic workup. In this workup ISAC 112 is used as a third-line diagnostic tool for the assessment of complex cases and care has to be taken when comparing multiplex and singleplex results.

### Funding

This work was supported by a research grant from Thermo Fisher Scientific/Phadia GmbH, Freiburg, Germany.

### Conflicts of Interest

AO, IS, MB, and JHM are/were employees of Thermo Fisher Scientific.

KB, JR, and TJ have received speakers’ fees from Thermo Fisher Scientific.

### References


Manuscript received April 14, 2014; accepted for publication, July 14, 2015.

Johannes Huss-Marp
Allergy Research Group
Department of Dermatology
University of Freiburg
Hauptstr. 7
79104 Freiburg, Germany
E-mail: huss-marj@web.de