Recommendations for the Use of In Vitro Methods to Detect Specific Immunoglobulin E: Are They Comparable?

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Abstract

Total and specific immunoglobulin (Ig) E can be detected in vitro using several commercially available methods. The largest share of the global market for these methods is held by the ImmunoCAP technique (Thermo Fisher, previously Phadia), Immulite (Siemens), and Hytec-288 (Hycor).

Most comparative studies examine Immulite and ImmunoCAP, which differ methodologically but use similar units of measurement relative to the same standard of total IgE (WHO IgE Standard 75/502). Despite their similarity, these kits differ in their quantification of specific IgE, which varies depending on the allergen studied. Thus, specific IgE results obtained with ImmunoCAP and Immulite are not interchangeable. It is important to bear this in mind, especially when determining cutoff points as predictors of a response to oral challenge with specific food allergens. The method used in practice must be the same as the one in the publication guiding clinical decision making.

We analyze differences between ImmunoCAP and ISAC microarray, 2 methods from the same manufacturer used to detect IgE to specific proteins (purified or recombinant). The results show that the IgE values obtained with ImmunoCAP are not equivalent to the corresponding values obtained with the ISAC microarray system.

Key words: Allergy diagnosis. IgE. Techniques. Enzyme immunoassay. Microarray

Resumen

Existen disponibles el mercado distintos métodos para la detección de la IgE total y específica. Los métodos con mayor cuota de mercado son método ImmunoCAP deThermofisher (anteriormente Phadia), Immulite de Siemens y Hytec-288 de Hycor.

La mayoría de los estudios comparativos se han realizado con Immulite e ImmunoCAP, que si bien difieren metodológicamente, emplean similares unidades de medida relativas al mismo estándar de IgE total (IgE Estándard OMS 75/502). Aunque estas técnicas estiman la cantidad de IgE total de forma similar, difieren en la cuantificación de la IgE específica. Se ha observado que estas diferencias varían en función del alérgeno al que se une la IgE específica. De esta forma, el resultado de la IgE específica para un alérgeno concreto obtenido por ImmunoCAP y por Immulite no son equiparables. Es importante tener en cuenta esta realidad, especialmente en el caso de puntos de corte determinados como predictores de la respuesta a una provocación oral con un alimento. El método empleado en la práctica debe ser idéntico al publicado como predictor.

También analizamos las diferencias en la determinación de IgE frente a proteínas específicas (purificadas o recombinantes) por la misma casa comercial pero empleando distintas tecnologías, ImmunoCAP y micromatriz ISAC. Los datos demuestran que los resultados obtenidos por ImmunoCAP para la IgE específica no son equivalentes a los obtenidos mediante la micromatriz ISAC.

Palabras clave: Diagnóstico alergológico. IgE. Técnicas. Enzimoinmunoanálisis. Micromatriz.

Introduction

In vitro quantification of specific immunoglobulin (Ig) E combined with skin tests constitutes the cornerstone of diagnosis of allergic diseases caused by a type I hypersensitivity mechanism. The presence of IgE indicates sensitization, which does not necessarily imply allergic disease. On the other hand, a negative IgE test result does not completely rule out allergic disease. Correct interpretation of test results often requires in-depth study, because the presence of serum specific IgE reveals sensitization that could have been induced by the allergen (genuine sensitization) or sensitivity caused by cross-reactivity, which may or may not be clinically relevant.

IgE was first identified in 1967 [1,2]. The first assay designed for its detection was the radioisotope-based radioallergosorbent test (RAST) [2], which came onto the market shortly afterwards in 1972 [3]. Since then, new IgE detection methods have been developed. Initially, only the complete extract of the allergen source was used. Today, we can also detect IgE to specific molecules that trigger an allergic reaction within a biological source, such as dust mite, pollen, or fruit.

Knowledge of the characteristics of currently available methods and of the main differences between them is necessary when tailoring the diagnostic test to a specific patient and correctly interpreting the results.

Characteristics of the Ideal IgE Detection Test

In recent decades, immunoenzymatic methods have become increasingly common and replaced radioisotope-based methods, thus removing the need for radioactive materials.

The most important qualities of a serum specific IgE detection method are accuracy, high sensitivity and specificity, reproducibility, standardization, and quality control [4,5]. Moreover, the technique should cover a broad range of allergens and be easy to use and automate, thus reducing the frequency of manipulation errors. The ideal method should be free of cross-reactivity with other immunoglobulins and also of IgE binding to other allergens. Thus, the system should be able to distinguish between genuine sensitization and cross-reactivity due to similarities between epitopes in allergens.

Optimal selection of allergens that bind to patient IgE is a constant object of research, as is the preparation of new allergenic molecules, ie, purified native proteins and recombinant proteins, which are free of carbohydrate cross-reactive determinants. The presence of these determinants in some vegetables and hymenoptera venoms leads to positive specific IgE results that can reveal clinically nonsignificant cross-reactions between pollen, latex, vegetables, and venoms [6,7].

Most Widely Used Commercially Available Methods for the Detection of Specific IgE

In recent decades, the development of new immunoassays (enzyme-linked immunosorbent assay [ELISA] and fluorometric assay), in combination with the possibility of automation, offers crucial technical advantages that increase accuracy (new calibration systems), sensitivity, and the speed with which results are available [5].

Over the years, in vitro diagnostic system manufacturers have generated procedures for the detection of antibodies, although some have withdrawn their products from the market. The largest share of the global market is now held by ImmunoCap (ThermoFisher, previously Phadia), Immulite (Siemens), and Hytec-288 (Hycor).

The method used in all 3 systems is similar to that of RAST. In the first step, the allergen (in fluid or solid phase depending on the assay) binds to serum specific IgE. In the second step, the specific IgE is detected by a second anti-IgE antibody conjugated to an enzyme. This enzyme catalyzes the transformation of a specific substrate with a quantifiable property (color or fluorescence). The different systems vary with respect to the matrix of the solid phase, the enzyme that binds to the detection antibody, and the reaction substrate. All 3 systems offer similar sensitivity and calibration with the same total IgE standard (WHO IgE reference standard 75/502), which is accepted as the gold standard calibration method [8]. Hence, the levels for specific IgE are extrapolated from a dose-response curve of total IgE according to a reference IgE standard. All 3 approaches are automated.

In the ImmunoCAP system, the allergen is covalently bound to a solid phase consisting of an activated hydrophilic polymer. The secondary IgE antibodies are bound to the enzyme β-galactosidase, which transforms added methylumbelliferyl-β-D-galactoside into a fluorescent product (4-methylumbelliferone). The fluorescence intensity depends on the concentration of the product and correlates with the IgE bound to the allergen. This correlation is established from a standard curve with a minimum of 6 concentration points.

The Immulite system, however, uses allergens bound covalently to soluble biotinylated polylysine polymers in a fluid phase that in turn binds to a streptavidin-covered polystyrene ball through a streptavidin-biotin complex. The secondary anti-IgE antibody is conjugated to alkaline phosphatase acting on an adamantyl-dioxetane phosphate ester substrate to emit a chemiluminescent signal. The correlation between the intensity of this signal and the levels of allergen-bound IgE is established from a standard curve of 7 concentration points.

The Hycor system uses an activated cellulose solid phase, which combines the allergen and alkaline phosphatase bound to the secondary antibody. The enzyme catalyzes the transformation of the *p*-nitrophenyl phosphate substrate in para-nitrophenol, a yellow compound. The intensity of this chromogenic signal corresponds to the levels of allergen-bound IgE on a standard curve of 5 concentration points (Figure 1).

A major difference between these methods is the source and quality of the allergenic extracts used. In a study of 283 allergic patients, Lee et al [9] evaluated agreement for specific IgE antibodies to 14 allergens (10 inhalant and 4 food allergens) measured with UniCAP 100 (which uses the same antigens as the current ImmunoCAP 250 and 1000) and Immulite 2000. The agreement observed for different extracts was highly variable: from 100% for birch pollen to 56.3% for shrimp.

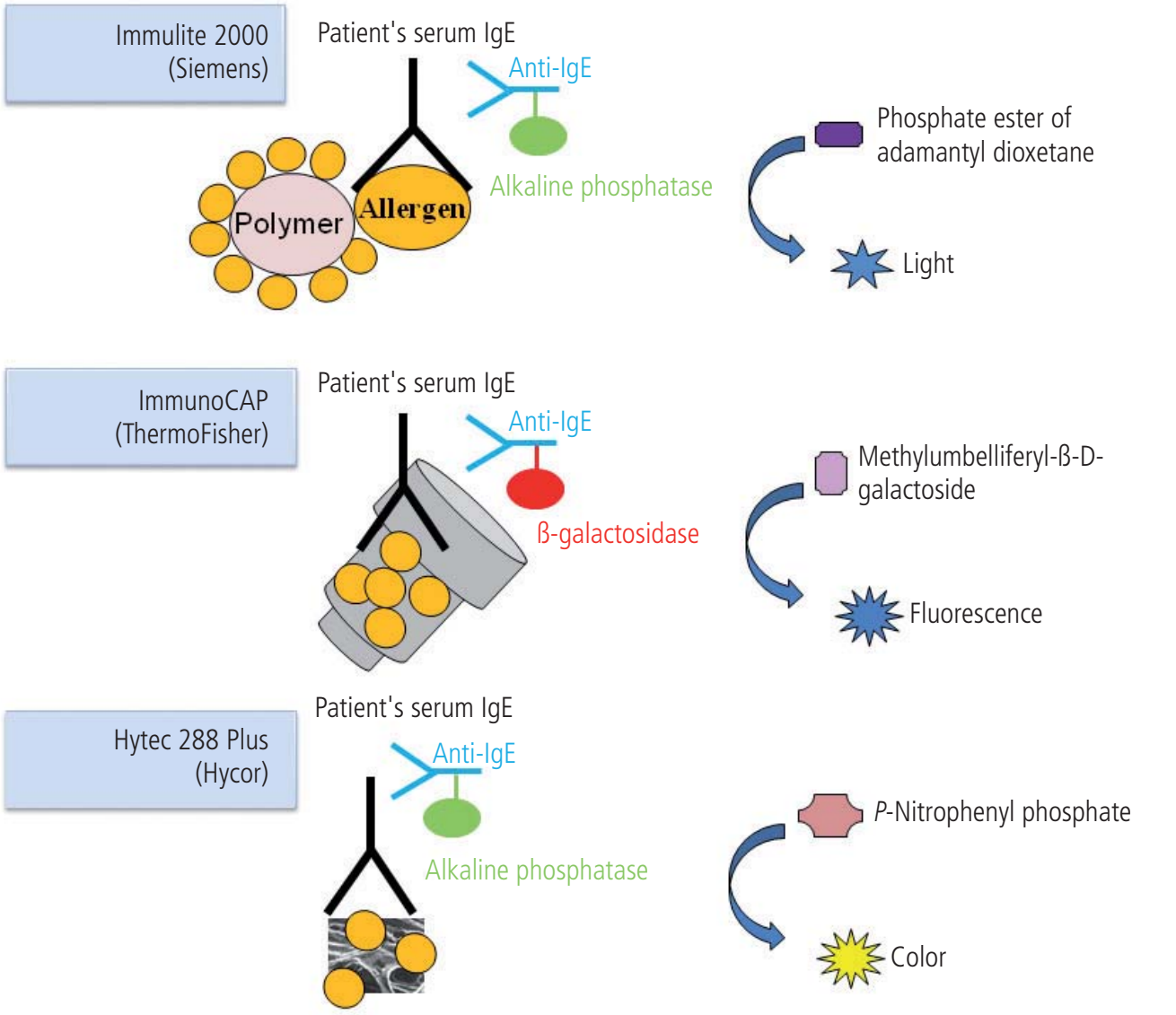

Figure 1. Methodology of the most widely used IgE detection methods. Ig indicates immunoglobulin.

Moreover, all methods attempt to minimize nonspecific binding of antibodies of other isotypes by supplying allergens bound to the solid or fluid phase in molar excess to the estimated concentration of circulating antibody.

Comparative Studies of the Most Widely Used Detection Systems for Specific IgE

Detection methods have been compared elsewhere [10,11]. However, previous studies are limited to ImmunoCAP and Immulite. Turbo-MP RIA (Hycor) has been compared with other techniques, although this radioactivity-based system has been replaced with the Hytec-288 ELISA (Hycor). Most studies on IgE detection methods show excellent results for parameters such as reproducibility, accuracy, and linearity. The similarities observed between some techniques, especially ImmunoCAP and Immulite, lead us to conclude that both methods are equivalent. Relevant differences, however, prevent them from being used interchangeably. Both ImmunoCAP and Immulite are calibrated using WHO IgE reference standard 75/502. The manufacturer's instructions state that 1 kU of total IgE with Immulite and 1 kU of total IgE with ImmunoCAP are each equivalent to 1 International Unit (IU) of total IgE. Hence, we could say that both systems are comparable. This might well be valid for total IgE, as proven by Wood et al [12] in a study with chimeric antibodies. The authors observed similar

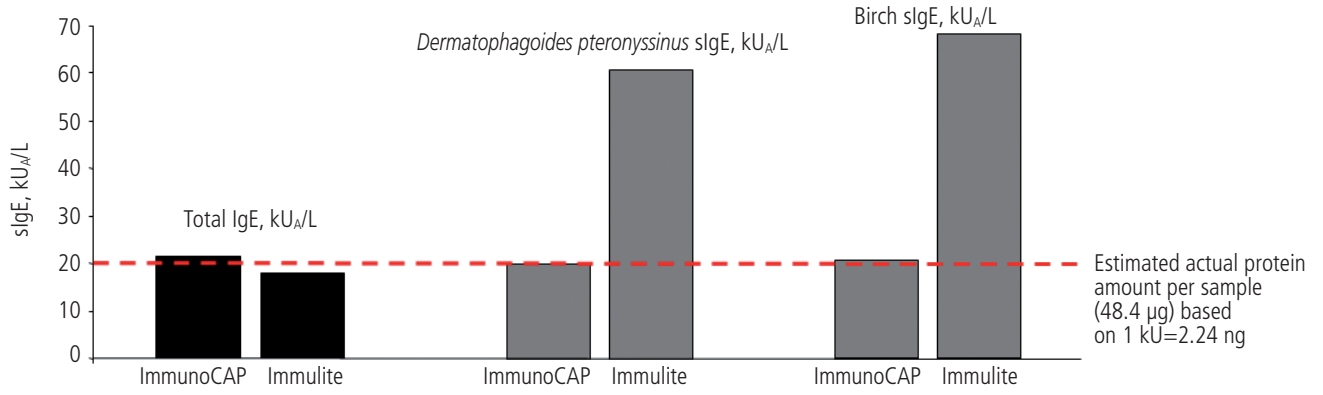

Figure 2. Total IgE levels and specific IgE to *Dermatophagoides pteronyssinus* and birch pollen determined with ImmunoCAP and Immulite for 48.4 µg of chimeric antibody to Der p 2 and Bet v 1. Modified from Wood et al [12]. sIg indicates specific immunoglobulin.

total IgE levels with ImmunoCAP and Immulite. However, after quantifying the chimeric antibodies, which recognized Bet v 1 and Der p 2 with the murine variable fraction through determination of specific IgE to birch and *Dermatophagoides pteronyssinus*, the results provided by the Immulite system were overestimated compared with those of the ImmunoCAP. Measuring total IgE offered similar results to IgE that was specific for birch and for *D pteronyssinus* using ImmunoCAP with different dilutions of the chimeric antibodies of Bet v 1 and Der p 2. On the other hand, measurement of IgE specific for birch and *D pteronyssinus* using Immulite yielded results that were 3-fold higher than those obtained with the same method for total IgE in the samples of Bet v 1 and Der p 2 (Figure 2) [12]. According to these data, the manufacturer of the ImmunoCAP says that the calibration for total IgE can be extrapolated to specific IgE [13]. ImmunoCAP units can even be converted to mass units (1 kU of IgE total=1 kU_A=2.42 ng) [14]. A recent multicenter study showed that the conversion factor between the kU_A of specific IgE obtained with ImmunoCAP and mass units was 3.23 ng [15]. Data on calibration of specific IgE compared to a standard and on conversion of kU_A to mass units are lacking for the Immulite system.

Specific IgE quantified using the Siemens method, however, seems to correlate better with the results of skin tests than IgE measured with ImmunoCAP. In a study of 9 allergens in 169 patients, the result for specific IgE obtained with Immulite correlated better with the skin test results than with the ImmunoCAP results [16]. The authors highlighted the greater sensitivity of Immulite without loss of specificity, as shown previously for allergy to hymenoptera venom [17]. Given that the detection limit in the ImmunoCAP was lowered from the previous 0.35 kU_A/L to the current 0.01 kU_A/L, new comparative studies on the sensitivity of both methods are necessary.

Although ImmunoCAP and Immulite do not provide equivalent results in the quantification of specific IgE, several authors have found a good correlation between IgE values obtained with both methods [8,9,18]. However, this correlation seems to vary depending on the allergen used for the quantification of specific IgE [9]. Even the same allergen can vary in its composition from one system to another and between batches in the same detection system. Therefore, studies comparing the results from the different methods using the same allergenic material or allergenic source are needed.

These methods are neither quantitatively nor qualitatively equivalent when defining whether IgE present is positive or negative [9,19]. Consequently, it is necessary to take into account the cutoff for IgE positivity, which must be appropriate for each method. This is especially important for the cutoff points used to predict clinical reactivity or response to a challenge test [20,21]. As these are of value in clinical decision making, the published cutoffs should not be applied by the allergologist when the method used for antibody detection is not the same as that used in the corresponding published study.

Comparison Between Common Detection Systems for Specific IgE and Protein Microarrays

The last decade has seen the arrival of new multiplex technology for the determination of specific IgE. Protein allergen microarrays [22] and flow cytometry [22] enable a more precise diagnosis by specifying the proteins responsible for the allergic disease. This diagnostic model offers higher diagnostic accuracy, especially in polysensitized patients. In addition, it provides useful information for the physician in the interpretation of cross-reactivity phenomena, prediction of severe reactions, and even tolerance of some food allergens. Finally, multiplex technology increases safety when prescribing immunotherapy since it is more accurate.

The usual detection methods also enable a molecular diagnosis to be made by replacing the complete extract of a biological source by a purified natural or recombinant allergenic component, thus enabling monocomponent quantitative determination of specific IgE. Once again, distinct specific IgE measurements obtained using different systems cannot be compared, even though the systems are manufactured by the same company and use the same allergen source.

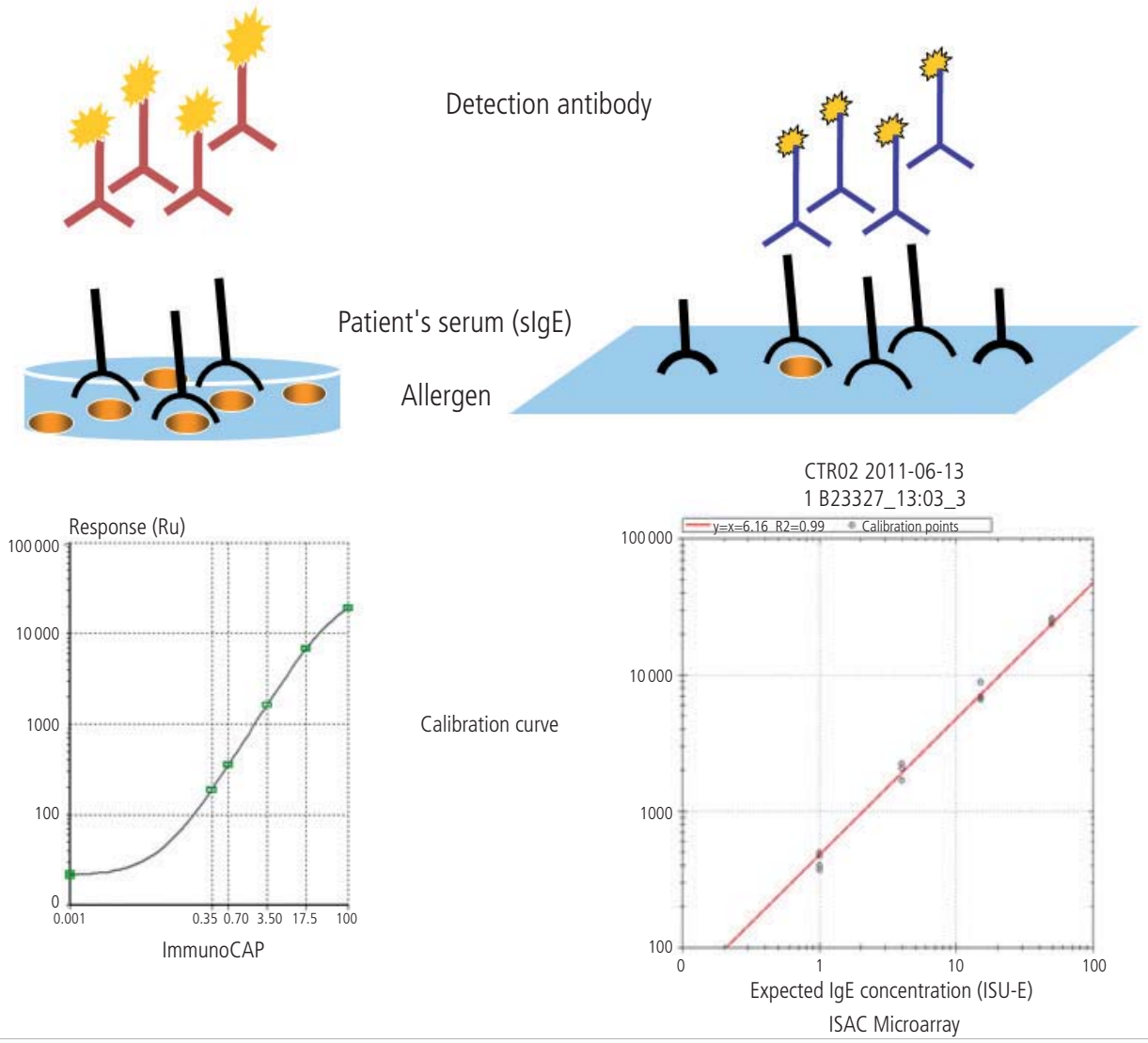

Figure 3. Methodology of specific IgE detection with components using ImmunoCAP and the ISAC microarray. sIg indicates specific immunoglobulin.

Such is the case of the commercial ImmunoCAP ISAC microarray and of the ImmunoCAP with proteins or allergenic components (both from ThermoFisher). Although both systems determine specific IgE to the same allergen, their results cannot be considered equivalent in quantitative terms, because each is subject to major technical differences in the antibody-antigen relationship, in adhesion of the antigen to the system base, in fluorochromes for IgE detection, and in calibration. The differences affecting the relationship between antibody and antigen arise from an excess of antigen in the ImmunoCAP system and an excess of antibody in the microarray system, which result in 2 noncomparable methods (Figure 3). While the ImmunoCAP is a quantitative method and can reliably detect a 2-fold reduction in IgE level in twice-diluted serum within the detection range of the assay, the microarray is semiquantitative, meaning that variations in the positive signal are expressed as an increasing series and in arbitrary units relative to the dose-response curve [23].

The correlation between the results from these 2 systems can also depend on the allergen. For example, after investigating 103 allergens, we found results for specific IgE that were not comparable for bromelain [24] or Der p 2 [25] with ImmunoCAP and ISAC microarray. However, the correlation between values for IgE to latex profilin [24] and Der p 1 [25] was high with both methods.

In qualitative terms (positivity and negativity), we also observed a variable relationship between the IgE results obtained with ImmunoCAP and with ISAC microarray. Agreement was 100% for allergens such as Gly m 4, Bet v 1, Fel d 1, Can f 1, and Der p 2, 35% for Asp f 1, and 40% for Phl p 7 [26]. In this sense, it is important to bear in mind that the cutoff must be appropriate for each method and allergen. We previously observed different optimal cutoffs for the ImmunoCAP and microarray system in the diagnosis of allergy to cypress pollen and to gramineae [27].

Conclusions

From a practical point of view and to avoid major errors in the diagnosis and treatment of allergic disease, it is important to bear in mind that the results obtained with different specific IgE detection methods are not equivalent.

Guidelines

- Results obtained with different methods for the measurement of serum specific IgE using currently marketed systems are not equivalent.
- The principal differences between IgE detection methods stem from the technology used (eg, phase in which the allergen is found, detection antibody, substrate for developing the reaction, and calibration systems) and from the source and quality of the allergenic extract. The results with Immulite could be higher than the results with ImmunoCAP when quantifying specific IgE to some allergens.
- In the case of some food allergens, it is not possible to interpret clinical reactivity or predict a response to a challenge test when the cutoff used is based on a specific IgE concentration obtained with a method that has not been scientifically validated.
- The specific IgE cutoff can differ with each allergen.
- Specific IgE results obtained with ImmunoCAP ISAC and ImmunoCAP with proteins or allergenic compounds (both from ThermoFisher) are not quantitatively equivalent, even when the same allergen source is used.

Acknowledgments

MJG, BG, LM, PG, and MLS are supported by grant RD07/0064 and RD012/0013 from the Spanish Research Network on Adverse Reactions to Allergens and Drugs (RIRAAF: Red de Investigación de Reacciones Adversas a Alérgenos y Fármacos) of the Carlos III Health Institute.

The authors would like to thank Juliane Chaccour for translating the original manuscript.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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