

Allergen Microarrays for Component-resolved Diagnosis

Marianne Brill

Introduction into ImmunoCAP ISAC® technology

The ISAC technology was originally developed and marketed by the biotech start-up company VBC Genomics Bioscience Research LLC (Vienna, Austria) [1–6]. Since 2008, the diagnostic product is available under the brand name ImmunoCAP ISAC® and distributed by Phadia. Since 2009 VBC Genomics LLC is a 100 % Phadia owned company.

ImmunoCAP ISAC (Immuno Solid-Phase Allergen Chip) is an *in vitro* diagnostic test for semi-quantitative determination of allergen-specific IgE antibodies in human serum or plasma. ImmunoCAP ISAC testing is envisioned as the tool for component-resolved allergy diagnosis in atopy screening. The test is designed for health care professionals in clinical laboratories, and test results are to be used in conjunction with clinical findings.

Based on modern protein biochip technology, ImmunoCAP ISAC is a miniaturised immunoassay platform that allows for multiplex measurement of allergen specific IgE antibodies (sIgE). The allergen chips are produced by ultra-low volume dispensing systems, which print several hundred spatially separated drops of allergen solution onto an area of less than a square centimetre. To detect allergen-specific IgE antibodies, this protein array of allergen components is incubated with a drop (20 µl) of human serum or plasma (Fig. 1). Binding of sIgE to immobilized allergen components is then detected by the addition of a secondary fluorescence-labelled anti-human IgE antibody. The procedure is followed by image acquisition using a microarray scanner. For signal quantification, images are analysed with proprietary software (MIA – Microarray Image Analysis Software). To obtain sIgE concentrations, signals are calibrated by the software and reported in ISAC Standardized Units (ISU). ISU are indirectly linked to a WHO reference standard for human IgE. The results are semi-quantitative and classified in four classes. The measuring range covers approximately

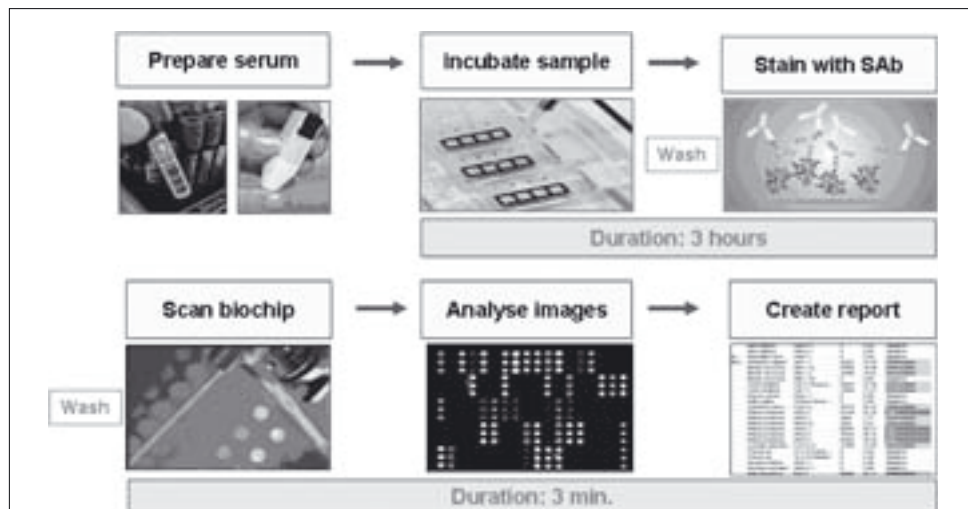


Fig. 1. Processing of ImmunoCAP ISAC during serum analysis for detection of allergen-specific IgE

0–100 kUA/l IgE when compared to Phadia’s “ImmunoCAP Specific IgE” test. The software generates a test report listing the sIgE results for each allergen.

Currently, the ImmunoCAP ISAC comprises a panel of 103 purified recombinant or natural allergen components, covering 47 different allergen sources. The panel includes major cross-reactive allergen components known today, such as profilins, nsLTPs and PR10 proteins, as well as a CCD-marker. The dataset obtained from one ImmunoCAP ISAC test represents the patient’s individual allergen sensitization profile. This information contains more than the sensitization status for the 47 allergen sources represented on the chip. The pattern of specific IgE antibodies detected in a sample also reveals the molecular pattern of the sensitization – according to the principle of component-resolved diagnostics (CRD [7,8]).

Component-resolved Diagnosis

The standard products used for *in vitro* (specific IgE) or *in vivo* (skin prick) allergy testing are based on allergen extracts prepared from biological raw materials. Allergenic sources contain allergenic and non-allergenic compounds. Amongst the allergenic compounds, there are unique allergens (markers for genuine sensitization for the particular allergenic source) as well as cross-reactive allergens (panallergens) such as profilins, which are present in most pollen and plant derived food [9–11]. Due to the presence of such cross-reactive allergens, extract-based testing can lead to false positive or misleading test results – even the allergen source might not be identified correctly. Because of their heterogeneity and the frequent instability of individual compounds, allergen extracts are difficult to standardize [12,13]. Consequently, diagnostic products from different manufacturers can result in irreproducible diagnostic outcomes providing low specificity. Obviously, it is not possible to

determine the disease-eliciting allergen molecules by extract-based testing. Extract-based allergy testing thus provides a poor decision basis for therapy, especially for specific immunotherapy (SIT).

However, with the increasing availability of purified molecular allergens (purified natural or recombinantly produced) comprehensive component resolved diagnosis (CRD) becomes feasible and paves the way for patient tailored, highly specific immunotherapy in the future [7,8]. In order to implement CRD for a broad panel of allergens, multiplex technology for *in vitro* testing is essential. Skin prick testing – which is widely used as a cheap, quick, and easy method for allergy diagnosis – is not applicable for CRD on a comprehensive basis. Allergen components for *in vivo* use are not yet widely available, and the number of tests to be done on the skin is practically limited. The application of conventional, singleplex *in vitro* tests (such as ImmunoCAP Specific IgE) for component resolved allergen screening is limited by cost and (esp. in small children) by serum volumes needed. Hence, ImmunoCAP ISAC provides a cost- and sample-efficient platform to enable CRD to be applied as a screening tool.

The method therefore provides a basis for optimal design of individual patient treatment, in particular in view of the progress expected in immunotherapy with the onset of patient tailored component resolved immunotherapy (CRIT) [14,15].

Last but not least, knowledge about the molecular nature of an allergic sensitization can help doctors to estimate the risk of an allergic reaction. For example, the presence of specific IgE against cross-reactive carbohydrate determinants (CCD) is seldom associated with clinical symptoms, whilst sIgE against LTPs (non-specific Lipid Transfer Protein) is often associated with systemic and more severe reactions. Combined with knowledge about the characteristics of allergen proteins (such as heat stability or homologies for food allergens), CRD can also lead to practical help in handling exposure to allergens.

Test performance

To evaluate the performance of ISAC testing, ISAC results are generally compared with Phadia's ImmunoCAP system being the gold standard for *in vitro* allergy testing. For allergen components where component ImmunoCAP tests are available, results can be compared directly. A respective comparison for recombinant birch and grass allergen components has been published by Deinhofer et al. and demonstrated excellent correlations [16].

To compare ISAC results with extract-based ImmunoCAP testing, the sum of ISAC results for respective allergen components can be used. Woehrl et al. published such studies for five common aero-allergens – house dust mite, cat dander, birch, grass and mugwort pollen [17]. The results demonstrate that for allergen sources, where an adequate repertoire of components is represented on the chip, ImmunoCAP ISAC and ImmunoCAP results compare very well in terms of sensitivity and specificity (e.g. timothy grass, birch, cat). However, lower sensitivity of ISAC testing was found for allergen sources where the set of components was presumably

incomplete, such as for house dust mite represented by *Der p 1* and *Der p 2* on the ISAC. Such results indicate the existence of further allergenic proteins, which are relevant for diagnosis but not (yet) included on the chip.

Further applications – Outlook

Beyond its commercial use, the versatile ISAC technology can be applied in research projects. In the EuroPrevall Project (www.europrevall.org) for example, ISAC testing enables the screening of a large number of samples for specific IgE against a novel panel of food allergen proteins.

In principle, the use of different fluorophors and detection antibodies in ISAC assays allows for testing of multiple parameters in parallel, such as for allergen-specific IgG and IgG subclasses. By measuring non-IgE allergen-specific antibodies, the ISAC technology can be used in research addressing allergen-specific antibodies other than IgE, or to monitor sIgG1 and/or sIgG4 for allergen components during specific immunotherapy.

Eventually, the comprehensive datasets of component-resolved sensitization profiles obtained from ISAC testing in patient screening are envisioned to be evaluated in a larger context. Combining the molecular ISAC data with other patient data – such as data on genetic factors, additional serum markers, place of birth and residence, personal, clinical and family history – should contribute to improve personalized allergy treatment and to progress in understanding of the disease.

References

1. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Cramer R, Duchêne M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Müller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Müller MW, Valenta R. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; 16: 414–416.
2. Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, Valenta R. Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy* 2003; 33: 7–13.
3. Jahn-Schmid B, Harwanegg C, Hiller R, Bohle B, Ebner C, Scheiner O, Müller MW. Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003; 33: 1443–1449.
4. Harwanegg C, Hiller R. Recombinant allergen based approaches for the diagnosis of IgE-mediated type I allergies. *Clinical Laboratory International November Issue* 2004.
5. Harwanegg C, Hiller R. Protein microarrays in diagnosing IgE-mediated diseases: spotting allergy at the molecular level. *Expert Rev Mol Diagn* 2004; 4: 539–548.
6. Harwanegg C, Hiller R. Protein microarrays for the diagnosis of allergic diseases: state-of-the-art and future development. *Clin Chem Lab Med* 2005; 43: 1321–1326.
7. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999; 29: 896–904.
8. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomés A. Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* 2000; 106: 409–418.
9. Valenta R, Duchêne M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelman H, Kraft D, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175: 377–385.

10. van Ree R. Clinical importance of cross-reactivity in food allergy. *Curr Opin Allergy Clin Immunol* 2004; 4: 235–240.
11. Pauli G. Evolution in the understanding of cross-reactivities of respiratory allergens: the role of recombinant allergens. *Int Arch Allergy Immunol* 2000; 123: 183–195.
12. van Ree R. Indoor allergens: relevance of major allergen measurements and standardization. *J Allergy Clin Immunol* 2007; 119: 270–277.
13. van Ree R, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O, Villalba M, Durham SR, Becker WM, Aalbers M, André C, Barber D, Cistero Bahima A, Custovic A, Didierlaurent A, Dolman C, Dorpema JW, Di Felice G, Eberhardt F, Fernandez Caldas E, Fernandez Rivas M, Fiebig H, Focke M, Fötisch K, Gadermaier G, Das RG, Gonzalez Mancebo E, Himly M, Kinaciyan T, Knulst AC, Kroon AM, Lepp U, Marco FM, Mari A, Moingeon P, Monsalve R, Neubauer A, Notten S, Ooievaar-de Heer P, Pauli G, Pini C, Purohit A, Quiralte J, Rak S, Raulf-Heimsoth M, San Miguel-Moncin MM, Simpson B, Tsay A, Vailes L, Wallner M, Weber B. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy* 2008; 63: 310–326.
14. Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. *J Investig Allergol Clin Immunol* 2007; 17 (Suppl 1): 36–40.
15. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, Purohit A, Arvidsson M, Kavina A, Schroeder JW, Mothes N, Spitzauer S, Montagut A, Galvain S, Melac M, André C, Poulsen LK, Malling HJ. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2008; 122: 951–960.
16. Deinhofer K, Sevcik H, Balic N, Harwanegg C, Hiller R, Rumpold H, Müller MW, Spitzauer S. Microarrayed allergens for IgE profiling. *Methods* 2004; 32: 249–254.
17. Wöhrl S, Vigl K, Zehetmayer S, Hiller R, Jarisch R, Prinz M, Stingl G, Kopp T. The performance of a component-based allergen-microarray in clinical practice. *Allergy* 2006; 61: 633–639.

Discussion

Sager: Could you please comment on the cost of that chip system?

Brill: Well, that is the responsibility of our marketing department, but I think it is about 150 € per assay. If you send us your samples and we do it, it is 200 €. I think that is about the range.

van Ree: I am a bit confused about the statement of “semi-quantitative” in combination with one of your last slides, which looks pretty quantitative. If you compare the CAP system and your ISAC, it looks pretty quantitative. So what is it now: is it quantitative or is it semi-quantitative?

Brill: It is definitively semi-quantitative. For certain allergens that are of high quality, like birch allergens or the timothy grass allergens, the comparison is very good, but it is also because of the nature of the test is a heterologous calibration. The concentration values are generated by taking 10 calibration allergens and the calibration serum, which has been characterized by CAP measurement. So we know how much IgE specific for 10 compounds is in that serum. And this is run alongside the assay to generate the calibration curve. So, all the 103 different interactions are valued according to this calibration curve to give the IgE concentration, which makes it semi-quantitative. It is not like an ELISA where you would have your particular cytokine as a standard curve, because there is no way to make a standard curve for all 103 interactions.

van Ree: That means that the CAP system is also semi-quantitative?

Brill: No, I would not say so.

van Ree: Really?

Mari: You have no μg given on your report for the CAP testing. So it is definitely semi-quantitative if you do not express the result as a concentration.

Renner: As a matter of fact, it is not a direct assay but an indirect assay. So the readout is indirect and therefore it cannot be quantitative.

Brill: The correlation between a high titer in serum IgE and the clinical significance is not that relevant. You know, somebody could have a rather low titer and has severe symptoms, but that is another discussion.

Chapman: I think you missed out the cost of the chip reader when you mentioned the prize, right? It costs about 30,000 €. My other question is a rather simple one. A few years ago, I visited VBC while they were setting up this assay and I certainly liked the vision of the whole. But they were actually using glass slides and washing them. And it looked a little bit like an immunofluorescence assay where you used to set up and wash these slides, too. You may say that is not a problem for a routine diagnostic procedure, but if you are planning to do a study of 10,000 patients and measure all these allergens, then, at a practical level, it does become an issue. So, I was just wondering has there been progress in there?

Brill: Yes, for the EuroPrevall Project we are just having a robot implemented which is doing the washing in a microtitre format.

Chapman: Okay. And what is the cost of the robot?

Brill: It is around 30,000 €.

Mari: May I give you some figures on that? We are close to 31,000 tested patients. And the main problem is automation, because we have some quite nice variability depending on the operator. And it takes time to have people there doing the washing and dispensing the serum or the secondary antibody and so on. So we are all looking for the robots because it will save time. Not money at the beginning, but no one of the company producing lab instruments will give you an instrument for free. So that is the basic cost for any testing in the lab. And I think there are many other aspects of this multiplex testing of molecules, but that cannot be targeted now. That should be discussed, but there is no more time.