

Microarrayed recombinant allergens for diagnostic testing

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The development of protein microarray-based immunoassays and the availability of recombinant allergens have, to a significant extent, emerged together over the past decade. Their long-anticipated wider application to allergy diagnosis has recently begun to accelerate. This review discusses some of the strengths and weaknesses of molecularly defined allergy testing and the microarray platform. Several recent applications of microarray assays to allergy testing are also summarized. Promising findings, particularly in the context of food and latex allergy, point to the potential for greater resolution between clinical reactivity and asymptomatic sensitization with this platform. (*J Allergy Clin Immunol* 2011;127:843-9.)

Key words: *Microarray-based immunoassay, microarray-based allergy testing, component-resolved diagnostics, molecular allergy diagnosis*

Although the limitations of sensitivity and specificity are inherent features of medical diagnostics, the correspondence between clinical *phenotypes* of IgE-mediated allergic diseases

Abbreviations used

CRD: Component-resolved diagnostics
ISAC: Immuno Solid phase Allergen Chip
SPT: Skin prick test

and the widely available measures of IgE sensitization is particularly poor.¹ When compared with provocation testing, especially when the history of exposure is limited, the performance of either skin testing or specific IgE-binding assays with whole allergen source extracts is weakly specific, and although generally sensitive, these also result in false-negative responses for a significant subset of patients.

To cite some recent evidence, a large, unselected, population-based evaluation of peanut sensitization by means of skin testing revealed a prevalence of 8.6%,² whereas the prevalence of clinical peanut allergy during the same time period was approximately 0.5% to 1%,³ suggesting a false-positive rate of approximately 90%. This rate is likely to be lower among younger children; however, in a study of 1072 children 4 to 5 years of age, the false-positive rate was still approximately 35%.⁴ High rates of false-positive results are found on provocation testing with aero-allergens, such as dust mite,⁵ as well. False-negative results are also important clinical problems, particularly in some contexts in which the risk/benefit ratio strongly favors the use of immunotherapy if the diagnosis is confirmed (eg, venom allergy). There are also subjects with negative results on skin prick tests (SPTs) or traditional serum specific IgE testing who have organ-specific reactivity of the respiratory or gastrointestinal tracts on allergen provocation and evidence of "local" mucosal IgE

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Terms in boldface and italics are defined in the glossary on page 844.

production that might be pathogenic.⁶⁻⁹ It remains to be seen whether in these subjects the use of refined allergen testing might improve sensitivity.

The utility of a diagnostic test is affected by its technical performance, as well as the relationship of the **biomarker** being measured to the clinical phenotype being evaluated. Beyond the fact that current widely used **solid-phase binding assays** are based on the use of whole allergen source extracts, it is not within the scope of this review to discuss the technical performance of existing assays, but both this and assay features that might influence correspondence to functional testing (eg, skin tests) and biological phenotype have been recently reviewed.¹⁰ Factors that have been identified as relevant to the performance of allergen-specific IgE measurement as a biomarker of immediate hypersensitivity include its **affinity**,^{11,12} fine specificity,¹³ **clonality**,¹⁴ and specific activity (ratio to total IgE).¹⁵ Non-IgE factors, including specific IgG, variations in effector cell reactivity, and end-organ sensitivity to mediators of allergic inflammation, will also affect that relationship.

MOLECULAR ALLERGY DIAGNOSIS: RATIONALE, ADVANTAGES, AND POTENTIAL PITFALLS

These biological variables of specific IgE are only meaningful in the context of defined allergens. Although at epithelial interfaces the immune system is confronted with complex, aggregated multimolecular structures, the structures or bioactivities of which can influence the immunologic response,¹⁶⁻¹⁹ the specificity of the adaptive immune response is at the submolecular level. Therefore the limitations of using the allergen-specific IgE response to an antigenically complex whole extract as a disease biomarker are evident, as has been pointed out by others for many years.²⁰ These limitations include the inclusion of irrelevant molecules, the presence of highly cross-reactive allergens and IgE **epitopes**, the limited representation of some allergenic molecules by abundance and perhaps solubility, and the potentially large variability

between preparations of crude extracts. This rationale has spurred the development of molecular or component-resolved diagnostics (CRD; a term interchangeable with molecular allergy diagnosis that refers to the use of individual allergen molecules to characterize patient humoral [usually IgE] immunity). At this time, one company (Phadia, Uppsala, Sweden) has brought CRD to market as either panels of selected (recombinant and purified native) allergens that can be tested on a fluorescence enzyme immunoassay platform (ImmunoCap) or a microarray-based assay (Immuno Solid phase Allergen Chip [ISAC]) of greater than 100 molecules.²¹ This is now widely available in Europe; however, in the United States it has not yet been approved for use by the US Food and Drug Administration and is available only as a research tool.

Currently almost 1000 defined proteins are known allergens, falling into 173 families containing 234 recognized protein family domains (approximately 2.0% of the >11,000 that have been described).²² This knowledge base forms a sort of "information ceiling" over the effort to define allergy diagnosis on a molecular level, but current testing is a log order of magnitude below this for several reasons. The infrastructure to recombinantly express and purify multiple allergens and verify their structural conformation is considerable and, along with intellectual proprietary and regulatory issues, represents a significant barrier to their widespread availability and use. There is also an interdependent relationship between the incentive to produce recombinant allergen-based tests and the discovery of their useful application that has slowed the pace of this technology being adopted more broadly. Few groups of researchers are independently cloning and expressing panels of allergens, although once those panels are available, many groups are well positioned to test their usefulness. Those allergens that have been incorporated into molecularly defined tests are those that have been identified in earlier work, often in the early stages by means of SDS-PAGE/**Western immunoblotting**, to be either specific to disease states or highly nonspecific and broadly cross-reactive (eg, **profilins**). This work is likely to be

GLOSSARY

AFFINITY: An attractive force between substances or particles that causes them to enter into and remain in chemical combination. This is in contrast to avidity, which refers to the rate of formation of an antigen-antibody complex.

BASOPHIL ACTIVATION TEST: A test based on flow cytometric demonstration of an altered membrane phenotype of basophils after stimulation by cytokines, allergen exposure, or both.

BIOMARKER: A distinctive biologically derived indicator of a process, event, or condition.

CLONALITY: Cells or antibodies that express identical receptors for the antigen.

EPITOPE: A molecular region on the surface of an antigen capable of eliciting an immune response and of combining with the specific antibody produced by such a response.

LIPID TRANSFER PROTEINS: Allergenic proteins found in multiple different plant species, including grasses and fruits.

MICROARRAY: A supporting material (eg, plastic slide) onto which numerous molecules or molecular fragments, usually of DNA or protein, are attached in a regular pattern for use in biochemical or genetic analysis.

PHENOTYPE: The observable properties of an organism that are produced by the interaction of the genotype and the environment.

PROFILIN: An actin-binding protein that is present in birch pollen, as well as fruits and vegetables. Profilins are responsible for the celery-mugwort-spice syndrome, as well as the oral allergy syndrome to apple, pear, carrot, celery, and potato, in patients allergic to birch.

PROTEOMICS: Applying the techniques of molecular biology, biochemistry, and genetics to analyzing the structure, function, and interactions of proteins.

RECEIVER OPERATING CHARACTERISTIC ANALYSIS: A plot of the true-positive rate against the false-positive rate for the different possible cut points of a diagnostic test.

SOLID-PHASE BINDING ASSAY: Material is used to capture antigen or immobilize antisera for subsequent reaction with secondary labeled antibody. The cellulose surface is the solid phase of the ImmunoCAP assay.

TROPOMYOSIN: A muscle protein that is the major allergen in shrimp. This protein can also be allergenic in other invertebrates, such as house dust mites and cockroaches.

WESTERN IMMUNOBLOT: An assay that allows for the identification of specific proteins in complex mixtures (eg, allergen extracts) by means of size-based separation.

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biased toward abundant molecules and might be missing important allergens from fractions of whole allergen that have been neglected (eg, by virtue of being less hydrophilic).²³ Challenges of using recombinant-based assays include difficulties expressing important posttranslational modifications of allergens, attaining the fully native tertiary structure (eg, the pectate lyase allergens, such as Amb a 1²⁴), or both. Finally, the inherent complexity of profiling individual patients' allergen reactivity to dozens of allergens has led to calls for algorithms to distill these rich data sets into clinically useful findings before these assays become widely used, lest we be drowned in a sea of information.²⁵

MICROARRAY PLATFORM: RATIONALE, ADVANTAGES, AND POTENTIAL PITFALLS

Botstein and Brown pioneered the application of large arrays of nucleic acids robotically printed on glass slides to profile and contrast the transcriptional activity of human cells in various disease states (eg, normal vs neoplastic).²⁶ This creative triumph led to an entirely new field of genomics and spurred the development of other applications (eg, analysis of DNA polymorphisms), eventually including efforts to target protein interactions²⁷ and the serological immune response to complex antigenic targets.²⁸

The primary advantage of adopting a multiplexed platform, such as a *microarray*, for component-resolved testing is that specific IgE to potentially thousands of allergens can be assayed in parallel with a small amount of serum. Because a traditional solid-phase assay typically requires on the order of 10 to 100 μ L per test, this might already begin to become practically limiting for profiling more than 100 tests in some patients (eg, children). By using a microarray-based assay, that same volume is more than sufficient to assay more than 1000 targets with controls and replicates. At the same time, much less allergen (approximately 1000-fold) is required per assay, and this is also a significant consideration because some allergens are difficult to express, purify, or both. Not surprisingly, the development of protein microarrays as an immunoassay platform has gone hand in hand with component-resolved diagnostics from the earliest stages of its use.²⁹ Phadia has brought that technology, initially developed by VBC Genomics and currently including 103 individual allergens from 47 species, to market as ISAC.³⁰ This assay uses a combination of purified natural and recombinantly expressed allergens. One of the potential strengths beginning to emerge from this more comprehensive profiling of allergic sensitization lies in the recognition of individual patterns of IgE reactivity to protein families with homologs across plant or animal species (eg, *tropomyosins*, *lipid transfer proteins*, profilins, and the pathogenesis-related protein Bet v 1 family).^{24,31-33}

Many of the advantages of the microarray platform, including the small amount of serum needed, the statistical power of assaying replicates in parallel, and the ability to simultaneously use multiple readout parameters (eg, the use of different fluorochrome-tagged targets to allow detection of multiple immunoglobulin subclasses) are generalizable beyond the use of individual allergens as targets. Research applications of microarray technology for allergy diagnosis are reported that target either finer (epitopes) or coarser (biochemical fractions) resolution than that of the individual allergen component. Profiling sequential epitopes of 1 or more allergens with a library of overlapping peptides based on their primary structure has been used in an effort to refine the diagnosis of several food allergies.³⁴⁻³⁷ In the opposite

direction in terms of molecular resolution, Renault et al³⁸ have recently reported on the use of a microarray-based immunoassay to profile the IgG, IgA, IgM, and IgE reactivity to sequential whole food allergen biochemical fractions from 350 food ingredients most commonly found in the United Kingdom population. Neither of these applications will be discussed further here.

Despite these advantages, there are features of microarray-based immune assays that differ significantly from other solid-phase assays¹⁰ and might be limiting. The major extract-based solid-phase immunoassays on the market are designed to make the allergen nonlimiting, and therefore the binding of IgE is not competitive with other antibody classes. These assays give a good estimate of IgE concentration specific to the combined targets present in the extract used, but they will readily detect even low-affinity antibody that might have little to no clinical relevance. In contrast, for a microarray-based assay, the allergen on a molar basis is often likely to be limiting relative to specific immunoglobulin, leading to potential interference between IgE and other isotypes, principally IgG. How this ultimately affects diagnostic performance remains to be rigorously evaluated.^{39,40} In addition, significant assay variations related to the microarray surface, array printing methods, and probing conditions demand careful scrutiny of assay performance.^{41,42}

Other limitations are inherent to the complex relationship between IgE specificity and function. An elegant model system using human basophils sensitized with various Der p 2-specific IgE clones of defined specificity and affinity has been used to demonstrate that the affinity, clonality, and specific activity (specific IgE/total IgE) are individually integrated into effector cell reactivity.⁴³ As discussed above, positive correlations have also been shown with each of these variables of patients' allergen-specific IgE to outcomes of effector cell activity, clinical sensitivity, or response to omalizumab.^{11,14,15,37,44,45} Therefore the diagnostic power of any solid-phase assay, whether based on purified components or unfractionated extracts, will likely be limited because not all of these features of the IgE repertoire will be captured. Some attempts to combine the assessment of specificity, clonality, and/or affinity to improve assay performance are being made on the microarray platform.^{12,46} Alternatively, by using functional assays, such as *basophil activation tests*, in combination with allergen fractionation³⁸ or the use of molecularly defined components,⁴⁷⁻⁴⁹ the need to dissect each of these components might be effectively supplanted by allowing a cellular system to integrate them into a measurable response.

EARLY APPLICATIONS OF MICROARRAY-BASED MOLECULAR TESTING

There are still few reports on the clinical performance of allergen microarray-based diagnostics. Examples of these from several clinical contexts are briefly summarized here and listed for reference in Table I.^{24,48,50-55}

Aeroallergy

In 2003, the group at VBC Genomics that developed what would become the ISAC, reported a comparison of a limited recombinant allergen array to conventional testing using approximately 50 subjects with presumptive birch or timothy grass pollinosis.⁵⁰ Proteins (Phl p 1, 2, 5, and 6 Bet v 1 and 2) were

TABLE I. Applications of microarray-based molecular allergen assays

	Clinical problem	Allergens	Allergen source	Array used	Patients	Reference
Aeroallergens	Tree/grass pollen allergy	Phl p 1, 2, 5, and 6 and Bet v 1 and 2	Recombinant	In house	Fifty-one allergic (history and testing), 11 atopic control subjects	Jahn-Schmidt et al ⁵⁰
	Ragweed/ Mugwort Allergy	Amb a 1, 5, 6, 8, 9; Art v 1, 3, 4, 5, 6	Natural and recombinant	In house	Ten ragweed allergic (history and SPT); 9 mugwort allergic (history and SPT)	Gadermaier et al ²⁴
Food allergens	Peanut allergy/tolerance	Ara h 1, 2, 3, and 8; Pru p 3; Bet v 1; Phl p 1, 4, 5b, 7, and 12; CCD	Natural and recombinant	In house	One hundred eight peanut-sensitized subjects identified from a healthy cohort of 1085, of which 17 were proved to be allergic and 52 were proved to be tolerant; an additional 12 with peanut allergy were specifically recruited.	Nicolau et al ⁵³
	Baker's asthma	Six <i>Escherichia coli</i> -expressed wheat proteins selected with sera from patients with baker's asthma; Phl p 1, 5, 7, and 12	Recombinant	In house	Twenty-two patients with baker's asthma (history and provocation testing); 32 with wheat allergy (history and provocation)	Constantin et al ⁵⁵
	Cow's milk allergy	Bos d 4, 5.0101, 5.0102, 8αs1, 8αs2, 8β, and 8κ	Natural and recombinant	In house	Seventy-eight milk-sensitized subjects of varying ages and clinical features	Hochwallner et al ⁴⁸
	Oral allergy syndrome	Mal d 1	Recombinant	Commercial		Ebo et al ⁵⁴
Contact allergens	Latex allergy	Hev b 1, 3, 5, 6, and 8; CCD	Recombinant except nAna c 2 as CCD	Commercial	Twenty-two allergic (history and testing); 20 asymptomatic with positive serum IgE levels; 26 healthy control subjects	Ebo et al ⁵¹
	Latex allergy	Hev b 1, 3, 5, 6, 8, 9, 10, and 11	Recombinant	Commercial, customized	Fifty-two adult patients with latex allergy; 50 control subjects with venom allergy	Ott et al ⁵²

CCD, Cross-reactive carbohydrate determinants.

printed by using a contact method, depositing approximately 2 ng onto an in-house silanized glass surface. Patients' IgE was detected with a fluorophore-labeled secondary antibody. The dynamic range was extended significantly by scanning the slides at 2 different laser power settings and extrapolating to assign values to those out of the linear range on either one of the 2 settings. Similar dynamic ranges, sensitivities, and specificities in comparison with those of atopic nonallergic control sera ($n = 11$) were reported when compared with both the Phadia CAP-FEIA and an in-house ELISA. The concordance rates between the microarray and the FEIA were 100% and 94% (9/51 subjects with positive results to Bet v 1; 3 [1 by means of FEIA and 2 by means of array]/51 subjects with positive results to Bet v 2); the concordance between the CAP-FEIA to timothy grass and the combination of Phl p 1, 2, 5, and 6 was 90% (18-20/51 subjects with positive results). All negative controls were negative with both assays. Correlations between the assays were greater than 0.9. There was no provocation testing of the subjects.

A similar but larger study was conducted by Wöhrle et al,⁵⁶ in this case comparing the commercial ISAC with the Phadia CAP system for evaluation of dust mite, cat, birch, grass, and mugwort allergy. A total of 120 subjects with allergy defined by means of

history and skin testing were evaluated. No provocation testing was performed. Using the CAP as the standard, the authors concluded, using *receiver operating characteristic analysis*, that the microarray was comparable for evaluation of cat, birch, and grass pollen allergies but slightly less sensitive for detection of sensitization to dust mite and mugwort pollen. In the absence of an extrinsic gold standard, it is not possible to know whether the lower sensitivity in comparison with that of the CAP assay might inversely correlate with greater specificity.

Lack of provocation testing also undermines the conclusions that can be made by a subsequent comparison of patients with diagnoses of either primarily ragweed or mugwort allergy ($n = 10$ and $n = 9$, respectively).²⁴ Nevertheless, the study hints at the potential usefulness of microarray allergens from each of these weeds, which overlap extensively in the flowering season and therefore might present a diagnostic challenge when deciding on optimal immunotherapy. Such an assay might be able to discriminate between primary sensitization to the major allergen of one source with additional potentially irrelevant nonspecific lipid transfer protein- or profilin-based cross-reactivity versus true cosensitization, possibly requiring immunotherapy treatment with both allergens.

Latex allergy

One study on latex allergy is highlighted here.⁵¹ Latex allergy diagnosis is well known to be confounded by a high rate of false-positive results with conventional testing. Ebo et al⁵¹ compared 3 groups of subjects using either a commercial microarray or singleplexed CAP-FEIA to conventional latex extract: 22 subjects with a strong history of clinical immediate hypersensitivity to latex with positive SPT responses and CAP-FEIA results, 20 subjects with sensitization documented by CAP-FEIA with negative SPT responses and documented asymptomatic exposure, and 26 healthy control subjects. The sensitivity of either CRD-based test using the combination of recombinant Hev b 1, 3, 5, and 6.02 approached 80%. None of the asymptotically sensitized subjects, in contrast, demonstrated specific IgE reactivity to these molecules, whereas approximately 75% of them did react to the profilin Hev b 8. The microarray and singleplexed CAP-FEIA performed identically. A second study published at almost the same time revealed similar findings when evaluating subjects with latex allergy versus control subjects with venom allergy.⁵² These findings now need to be confirmed in a larger prospective study including more difficult cases (eg, clinically reactive with a negative SPT response or asymptomatic with a positive SPT response), but these studies clearly exemplify the promise that this methodology might be better able to distinguish clinical allergy from asymptomatic sensitization.

Food allergy

Yet another example of the promise of CRD with a microarray format comes from a 2010 study examining peanut allergy versus sensitization. Nicolaou et al⁵³ found that 11% (110/933) of children from a population birth cohort in the United Kingdom were sensitized to peanut at 8 years of age. Approximately two thirds of this group (66/91 fully assessed) passed oral food challenges, about one fifth were confirmed to be allergic (12 by means of a convincing history without challenge and 7 by means of challenge), and the remainder (n = 6) were deemed inconclusive. Peanut-specific IgE levels were significantly higher in the allergic group (supplemented by an additional 12 children with known peanut allergy), whereas the tolerant group had more allergic rhinitis, fewer known food allergies, and higher grass-specific IgE levels.

The authors found that the misclassification rate in this cohort using the widely accepted criteria of a peanut-specific IgE level of 15 kU/L or greater or an SPT wheal size of 8 mm or greater was approximately 15%. They reduced the overall misclassification rate to 7.4% using a capillary-flow membrane-based microarray containing purified native or recombinant Ara h 1, 2, 3, and 8; Phl p 1, 4, 5b, 7, and 12; Bet v 1; Pru p 3; and cross-reactive carbohydrate determinants. Ara h 2 was the single most predictive component for allergy versus sensitization in this population, a finding that is consistent with independent comparisons of the relative potency of peanut allergens.⁵⁷

Other interesting applications of microarray testing for food allergy have included a comparison of patients with birch allergy with and without oral allergy syndrome to apple,⁵⁴ characterization of cow's milk allergy,⁴⁸ and discrimination of baker's asthma from wheat allergy.⁵⁵

Venom allergy

Venom allergy is another important clinical presentation for which extensive cross-reactivity leads to high rates of nonspecific

sensitization, but in addition, false-negative findings with conventional testing are also common and clinically problematic. Two 2010 articles have demonstrated promise for improvement by using recombinant phospholipase A₂ and group 5 antigens,^{58,59} but these have yet to be widely evaluated on a microarray format. Api m 1 and 4 are currently included in the ISAC, but group 5 allergens are not.

IgE profiling

The application of allergen microarrays for IgE profiling of subjects with atopic diseases associated with broad sensitization, which is often of uncertain diagnostic utility, could also prove to be an area of important advancement in coming years. In a study of 20 adults with atopic dermatitis, Ott et al³³ found that they were sensitized to an average of 21 specific allergens. Many of them recognized cross-reactive allergens. Whether such profiling will prove to better inform our understanding of disease pathogenesis in this or other contexts (eg, asthma or allergic eosinophilic gastrointestinal disorders) remains to be seen.

FUTURE

A look at some newer ways in which microarray immunoassays are being used outside of allergy suggests some areas of more cutting-edge applications to our own discipline. As mentioned above, current allergen characterization is probably significantly biased to those molecules that are more abundant and water soluble. One way to at least partly address this limitation is exemplified by the approach reported by Cruz-Fisher et al⁶⁰ to comprehensively identify *Chlamydia* species antigens. They used systematic *proteomics* to construct a microarray comprised of all open reading frames present in the murine pathogen *Chlamydia trachomatis*. Of the 185 recognized antigens (from approximately 900 proteins screened), many known immunogens were "rediscovered," but in addition, more than 75 novel antigens were also identified. New and more sensitive methods of antibody binding detection^{61,62} and the use of printed microarrays as a platform for cellular assays are also promising developments with exciting potential future applications in our field.^{63,64}

In summary, the immediate future promises a rapid influx of new data now that microarray-based tests are becoming more widely available. This will allow many groups to design more and stronger studies to critically evaluate their diagnostic and prognostic power over existing test modalities, and we should learn a great deal about this for all of the areas highlighted above in the next couple of years.

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Microarrayed recombinant allergens for diagnostic testing

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Learning objectives: "Microarrayed recombinant allergens for diagnostic testing"

1. To understand the techniques used in immunology laboratories.
 2. To assess the reader's ability to comprehend the manuscript.
 3. To determine whether further investigation or research on the reader's part is necessary.
-

CME items

Question 1. Microarray methodology identified which isoform of the major peanut allergen as most predictive of peanut allergy and potentially helpful in distinguishing allergy from sensitization?

- A. Ara h 1
- B. Ara h 2
- C. Ara h 3
- D. Ara h 8

Question 2. Patients with which allergic disease often have positive skin test results to multiple allergens?

- A. asthma
- B. atopic dermatitis
- C. allergic eosinophilic gastrointestinal disorders
- D. food allergy

Question 3. Which of the following is a limitation of the whole-extract allergen-specific IgE response?

- A. inclusion of only relevant molecules
- B. absence of highly cross-reactive allergens and IgE epitopes
- C. overrepresentation of some allergenic molecules by abundance and solubility
- D. potentially large variability between preparations of crude extracts

Question 4. Recombinant-based assays can be limited by which of the following?

- A. lack of expression of important posttranslational modifications
- B. overreliance on native tertiary structure
- C. limited data for interpretation
- D. incorporation of cytokine signaling

Question 5. In subjects with latex sensitization documented by means of CAP-FEIA with negative skin prick test results and negative documented asymptomatic exposure, which of the following molecules would most likely demonstrate specific IgE reactivity?

- A. Hev b 1
- B. Hev b 5
- C. Hev b 6.02
- D. Hev b 8

Question 6. Which of the following venom antigens is currently included in the Phadia Immuno Solid phase Allergen Chip?

- A. phospholipase A₂ (Api m 1)
- B. Api m 10
- C. group 5 allergens
- D. bee antigen 7M

Question 7. Among the factors relevant to allergen-specific IgE as a marker of hypersensitivity is —

- A. glycosylation.
- B. T_H17 activity.
- C. IL-4 and IL-13.
- D. specific activity (ratio to total IgE).

Question 8. Non-IgE factors that are relevant markers of immediate hypersensitivity include variations in effector cell reactivity, end-organ sensitivity to mediators of allergic inflammation, and —

- A. specific IgA.
- B. specific IgG.
- C. specific IgM.
- D. specific IgD.

Question 9. Which of the following is true of microarray-based immune assays?

- A. They can readily detect even low-affinity antibody that might have no or little clinical significance.
- B. They require a larger amount of allergen per assay than traditional solid-phase assays.
- C. The allergen can be limiting relative to specific immunoglobulin, possibly allowing for interference between IgE and other isotypes (specifically IgG).
- D. They are functional assays.

Question 10. A study performed by Kopp et al demonstrated that a microarray was equally as sensitive as the Phadia CAP system in detecting IgE to which of the following?

- A. birch
- B. mugwort
- C. dust mite
- D. cockroach