A diagnostic approach to IgE-mediated food allergy: A practical algorithm

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ABSTRACT

A food reaction history is the basis of food allergy diagnoses. Several levels of food allergy diagnostic testing can confirm or refute the presence of food allergy. The choice of food allergy testing modality should be informed by the reaction history and determined by the testing goals. Testing modalities include skin-prick testing, in vitro specific immunoglobulin E testing, component-resolved testing, epitope threshold testing, and basophil activation testing. The goal of food allergy testing may be merely to confirm the diagnosis of food allergy or may be used to guide passive (avoidance) or active (allergen immunotherapy) management. The most appropriate diagnostic path should consider testing predictive value, the goal of the evaluation, patient and family food allergy anxiety, and cost. Peanut allergy testing provides an algorithm for testing pathways.

(J Food Allergy 6:15–20, 2024; doi: 10.2500/jfa.2024.6.240007)

Generally, a medical history provides the primary basis for medical diagnoses, with only minor contributions derived from the physical examination and diagnostic testing.[1] However, because of the inherent uncertainties in the history of food allergy reactions and, typically, a normal physical examination at the time of the office visit, testing plays a much more important role in immunoglobulin E (IgE) mediated food allergy diagnoses. Nevertheless, a high-quality, food allergy–focused history is necessary to establish the clinician's estimate of the pretest probability of food allergy because that estimate is crucial in assessing the results of food allergy testing.[2]

GOALS OF FOOD ALLERGY DIAGNOSTIC TESTING

Although the primary goal of food allergy diagnostic testing (FADT) is to confirm or refute a food allergy, some FADT modalities may be used to identify a patient's reaction eliciting dose or maximum tolerated dose of the allergenic food. This information can be very helpful in food allergy management by simplifying avoidance for those with a high eliciting dose or identifying a starting point for oral immunotherapy (OIT). FADT is also helpful in assessing the response to active treatment of food allergy with OIT, sublingual immunotherapy, and other treatments currently under study.

Ideally, the patient will present with a history of a recent food reaction strongly suggestive of IgE-mediated disease, and the confirmatory testing will be straightforward. In reality, patients present with a food allergy concern based on a history of a recent food reaction suggestive of IgE-mediated disease, a history of a remote food reaction suggestive of IgE-mediated disease, or a history of a clinical problem that may or may not be directly associated with the ingestion of a particular food and may or may not have the characteristics of an IgE-mediated food allergy. Often, there has been a positive test with no history of ingestion or reaction, or simply parental anxiety that may be based on a family history of food allergy or another factor. The patient's presenting history and the goals of the evaluation should determine the choice of food allergy testing modality. When the patient's history is not compatible with a diagnosis of IgE-medicated food allergy, it is incumbent on the clinician to dissuade the patient or family from inappropriate testing. The potential goals of FADT are listed in Table 1.

FOOD ALLERGY TESTING MODALITIES

Although the most highly predictive FADT is double-blind placebo controlled food challenge (DBPCFC), blinded food challenges are resource intensive and potentially dangerous.[3] Consequently, other testing modalities have been developed as surrogate markers for IgE-mediated reactivity to a food. The element of food allergy testing common to all testing modalities is

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Funding was provided by the Eastern Food Allergy & Comorbidity Conference
Presented at the Eastern Food Allergy and Comorbidity Conference, January 7, 2024, Palm Beach Florida
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Table 1 **Goals of food allergy diagnostic testing**

Informing Avoidance Management Decisions	Guiding Food AIT
Making the diagnosis, yes or no	Determine the appropriateness of AIT
Identify food(s) that should be avoided	Identify a standard AIT starting dose
Strict avoidance	Assessing AIT risk factors
Less than strict avoidance	Threshold testing for low dose AIT
Precautionary allergen labeling - "May contain" and "Manufactured in a facility that also manufactures"	Support the use of a biologic immunomodulator

AIT = Allergen immunotherapy.

the fidelity of the food allergen in the test system compared with the native allergen that has elicited or could elicit a reaction. Each method of testing has its strengths and weaknesses, among them, the sensitivity and specificity of the test results and the integrity of the test allergen.[3]

MEASURING FOOD-SPECIFIC IgE BY SKIN-PRICK TESTING

Skin-prick testing (SPT), the mainstay of allergy practice, has the advantages of low cost, immediate results, and a high negative predictive value (NPV) (but not 100%).[4] The reliability of SPT is limited by operator and device variability. Because of a significant rate of false-positive results (50%), the positive predictive value (PPV) is not high.[4] Using a high cutoff, however, improves specificity. By using the prick-to-prick technique, SPT can be used on whole foods and is particularly helpful when the suspect allergen is a fruit or vegetable.[4] Because of the variability of commercial extracts for fish and shellfish, using the prick-to-prick technique may be particularly helpful if the testing result with a commercial extract is negative.[5] Prick-to-prick testing may identify an important allergic sensitivity to any food when the history is convincing but when the testing result by using commercial extracts is negative.[6]

MEASURING FOOD-SPECIFIC IgE BY USING *IN VITRO* TESTING

In vitro FADT is based on binding patient specific IgE (sIgE) to a food allergen in solution or bound to a solid matrix as shown in Figure 1.[7] *In vitro* testing has the advantage of high reproducibility and the ability to perform testing on patients who cannot stop antihistamines for SPT. It has a high sensitivity and specificity. The cutoff values chosen determine the PPV and NPV power of the test. Use of a low cutoff for positivity (*e.g.*, 0.35 kU/L) makes the test highly sensitive for food allergy but yields a high rate of false-positive results. A low cutoff helps exclude allergy to the test food. Although most commercial laboratories report the same range of values for all foods, the PPV and NPV of *in vitro* food testing vary by food. It is essential to explain this when discussing results with patients and parents. Reported PPV and NPV also vary by the

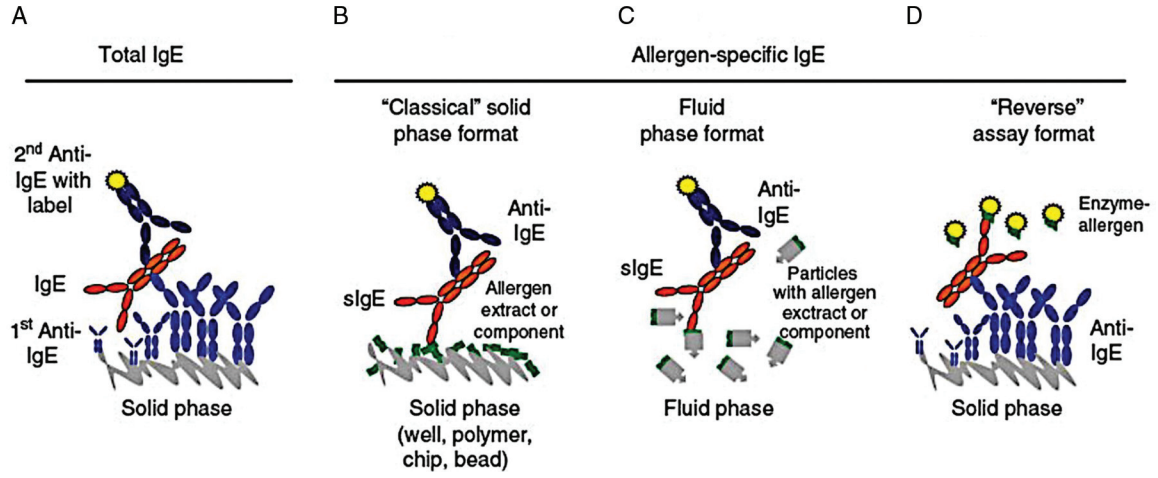

Figure 1. *Principal IgE assay formats. (A) General total IgE assay format. (B) "Classical" solid phase assay format for the detection of sIgE. (C) Fluid-phase assay format for sIgE. (D) Reverse phase assay format for sIgE. IgE = Immunoglobulin E; sIgE = specific IgE. (Reproduced from Ref 7.)*

population studied. The sIgE testing may be confounded by very high total IgE levels, such as those seen in patients with atopic dermatitis.[8]

COMPONENT TESTING

Foods are natural products that comprise a myriad of different proteins, only some of which stimulate the production of IgE antibodies capable of eliciting an allergic reaction. Testing for IgE directed at specific components of the allergenic food has better specificity than testing to a whole-food extract.[8,9] The most well-studied example is peanut testing, in which component-resolved testing identifies the IgE antibody against Ara h2, the primary allergenic protein that elicits an anaphylactic response; Ara h6, another high-risk protein; and Ara h8, a pathogenesis-related-protein-10 (PR-10) protein that is not associated with severe allergic reactions to peanut. Component-resolved testing has improved specificity compared with whole-food testing and is available for several allergens, particularly tree nuts.

Multiplex *in vitro* testing assays sIgE directed at a large panel of food allergens and their components.[10] Multiplex testing has the advantage of providing information about many foods and food components in a single test that requires only a small blood sample. It has the important disadvantage of providing information independent of the patient's history. By panel testing multiple foods with a single test, there is the risk of identifying clinically irrelevant sensitizations that, at a minimum, will require an explanation to the patient or parents, and may lead to otherwise unnecessary oral food challenges (OFC) or dietary avoidance. In this setting, the unnecessary dietary avoidance may increase the risk of developing IgE-mediated allergy to the avoided food.

PEANUT EPITOPE TESTING

The precise peanut epitope that elicits an IgE response that can trigger anaphylaxis has been identified. At the time of writing this article, testing for reactivity to the major allergenic epitope of peanut Ara h2 (Fig. 2) is the only commercially available epitope test.[11] This commercial test characterizes the patient's reactivity threshold as low, medium, or high based on the calculated likelihood of tolerating specific doses of peanut protein. Interpretation and explanation of peanut epitope testing requires understanding the peanut protein challenge dose reaction rates for each reactivity classification, as shown in Figure 3.[12]

BASOPHIL ACTIVATION TESTING

Basophil activation testing (BAT) uses a food allergen extract to stimulate the basophils in whole blood. The

Figure 2. *The structure of the major epitope of Ara h2. The primary amino acid sequence and conformational structure of the major epitope of Ara h2. (Reproduced from Ref. 12.)*

stimulated cells are assessed by using flow cytometry to detect the expression of the basophil activation markers CD63 and CD203c (Fig. 4).[13] Basophils can be activated by a single appropriate dose of the food allergen or a dose-response can be determined. The advantage of activating basophils with a range of doses is that the dose-response curve provides a rough estimation of the patient's eliciting dose. BAT has not been widely used clinically because of assumptions that basophil reactivity could not be accurately measured on samples > 4 hours old. However, Kim *et al.*[14] demonstrated that inducible basophil markers are stable for 48 hours *ex vivo.* The sensitivity and specificity of BAT exceeds 95%, which obviates the need for OFCs for most patients.[15] The major limitation of BAT is that ~10–15% of patients do not respond to BAT.[16]

FOOD CHALLENGES

The DBPCFC is the most reliably predictive food allergy testing modality against which all other procedures are measured.[4] The DBPCFC minimizes the impact of health-care team, patient, and companion biases during the challenge as well as psychologically based false-positive results. DBPCFCs are, however, resource intensive and costly. Single-blind challenges are an option, but informing the patient that a placebo controlled challenge is being done to avoid undermining the physician-patient relationship is essential. Open challenges are usually satisfactory, although some clinicians routinely use a placebo for the first dose.

The algorithm for OFC typically begins with a dose estimated to be below the patient's eliciting dose followed by administering escalating doses until a meal-sized portion of the suspect food has been consumed. Failure to provide a sufficiently large exposure to the suspect food can result in a false-negative OFC. From a practical clinical point of view, the OFC should

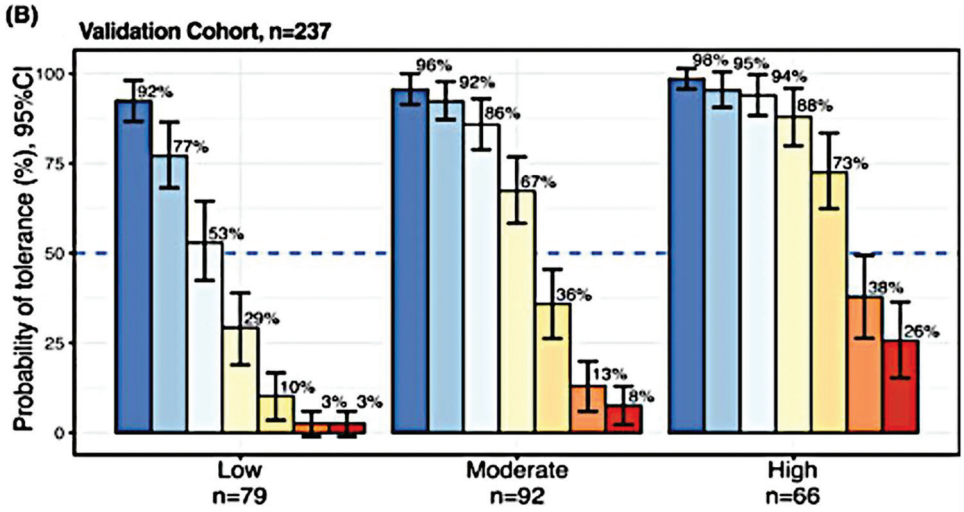

Figure 3. *Characterization of peanut threshold reactivities. The bar charts show the probabilities, with 95% confidence intervals (CI), of tolerance at each peanut dose for "low," "moderate," and "high" dose-reactivity groups in 237 validation subjects. The peanut protein doses represented in the bar graphs (reading left to right) are 4 mg, 14 mg, 44 mg, 144 mg, 444 mg, 1444 mg, and 4444 mg. (Reproduced from Ref. 12.).*

duplicate an everyday exposure to the food. When designing OFC, the clinician should be mindful of the total cumulative dose provided during the challenge. Depending on the goal of the OFC (see below), the challenge algorithm can terminate at a dose well below a meal-sized portion. In clinical studies, for example, if the entry criteria require a positive OFC of < 300 mg of allergen protein, there is no reason to dose with > 300 mg in the challenge. For some patients, a limited challenge to determine the eliciting dose (the lowest dose that will trigger a reaction) or to identify the patient's threshold dose (the maximum dose that can be tolerated without a reaction) may be appropriate. Although not strictly for food allergy diagnosis, proximity challenges, during which patients who report symptoms in the absence of ingestion or contact with the allergenic food are exposed to an open container of the allergenic food that is gradually moved closer to them can be life changing.[17]

GOALS OF FOOD CHALLENGES

A food challenge's goals should be defined before planning the challenge. Because of the cost of food challenges and the risk of anaphylaxis, the use of food challenges and the choice of the challenge algorithm should

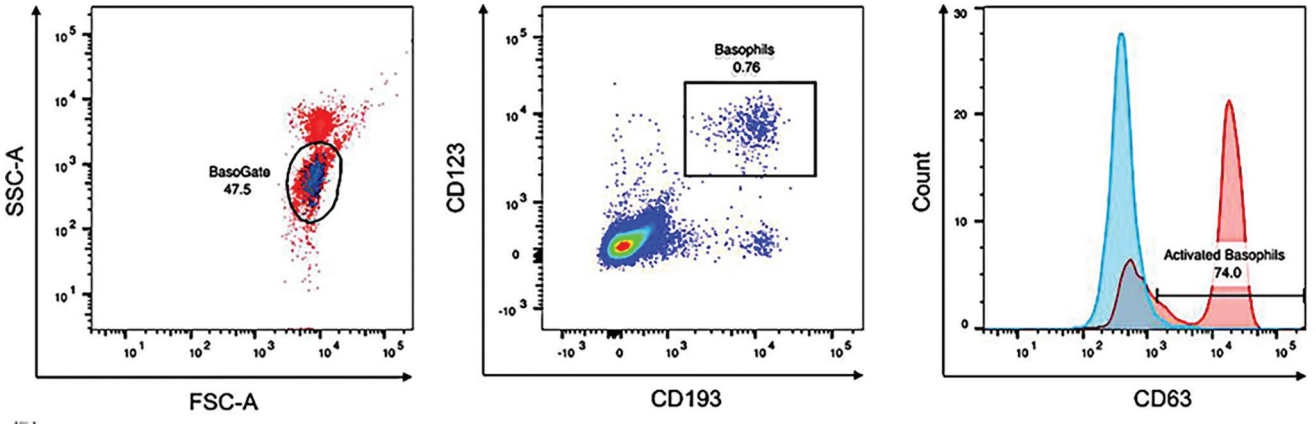

Figure 4. *Basophil activation testing by flow cytometry. Basophils were identified as SSClow CD123c^{+} CD193^{+} cells 45, 48: 1. Lymphocyte – monocyte gate on a FSC/SSC plot by using a logarithmic scale, 2. Doublet exclusion FSC-H versus FSC-A, then SSC-H versus SSC-A, 3. Gate on both markers simultaneously CD123 and CD193, 4. CD63 negative threshold was set to 2.5% and the positive population above that threshold was assessed. SSClow = side scatter low granularity; FSC = forward scatter; SSC = side scatter; SSC-A = side scatter area; FSC-H = ; FSC-A = forward scatter area; SSC-H = side scatter height. (Reproduced from Ref. 13.)*

Table 2 **Goals of food challenges**

Type of Food Challenge	Special Features and Objectives of Performing the Food Challenge
Double-blind placebo controlled	Confirm or refute food allergy Minimize potential bias in outcome assessment Minimize the risk of false-positive challenges due to anxiety
Open	Confirm or refute food allergy
Threshold dose	Determine the highest tolerated dose Inform counseling for food avoidance that is less than absolute Determine a starting dose for standard oral immunotherapy Determine a dose for low-dose, ultra-slow oral immunotherapy
Eliciting dose	Quality for research studies
Proximity challenge	Mitigate food exposure anxiety

be the subject of a shared decision-making discussion. The patient and family should control the final decision based on their goals and concerns. The health-care team must, however, fully inform the patient and family of the ramifications of their decision with regard to avoidance management, including the psychological and monetary costs of food allergen avoidance, and the implications of the potential challenge results for more active management. The goals of different types of food challenges are summarized in Table 2.

A PRACTICAL APPROACH TO FOOD ALLERGY DIAGNOSIS

Peanut was chosen as the prototypical allergenic food for this discussion because of the large amount of peanut testing and diagnostic data available. When extrapolating the algorithm to other foods, the clinician must be mindful of differences in each testing methodology's food-specific PPVs and NPVs. Evaluation of reports of the diagnostic value of testing modalities must consider the population on which the data are based because of regional differences in the prevalence of specific food allergies and apparent regional differences in sIgE responses.[4]

An algorithm for a peanut allergy diagnosis is presented in Fig. 5. Application of this diagnostic pathway must consider both the patient's history and his or her goals. The following examples of different patients illustrate the use of this diagnostic approach. There would be shared decision-making discussions with the

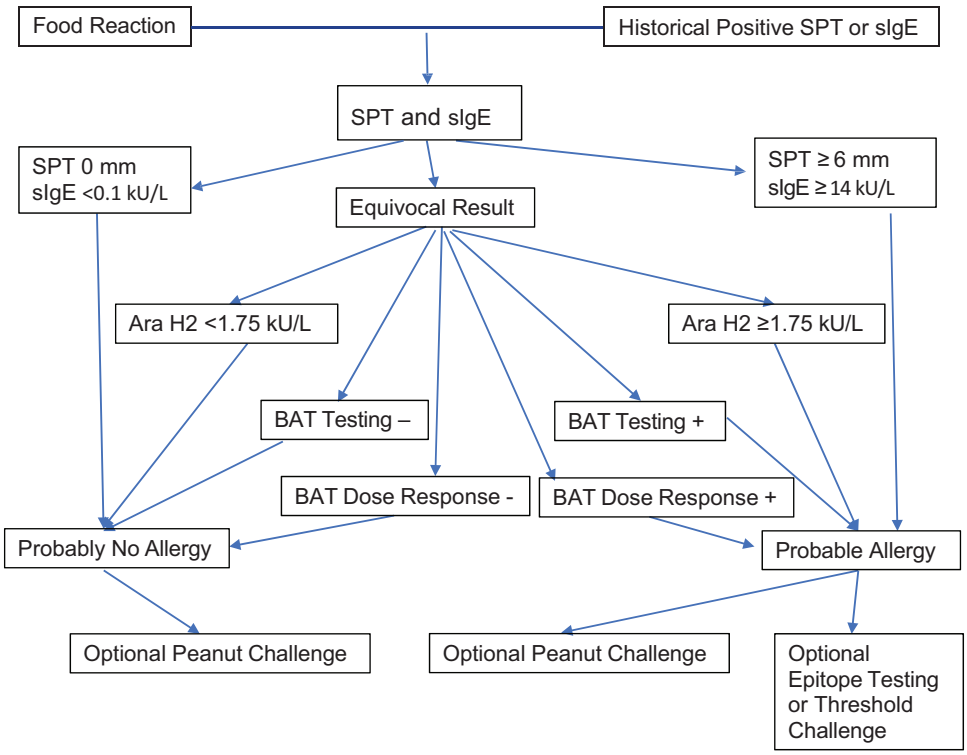

Figure 5. *Diagnostic testing algorithm for peanut allergy.*

family, but a recommendation is included for the sake of this discussion.

Case 1: A 6-year-old is evaluated because of a remote history of a weakly positive sIgE value but no history of ingesting peanuts. Recommendation: a negative SPT result or sIgE value is sufficient to allow a single-step challenge or home introduction.

Case 2: A 4-year-old experienced a grade 2 anaphylactic reaction on peanut exposure 3 months ago. The SPT result is 13 mm greater than the negative control. Recommendation: peanut allergy is confirmed. Additional testing is optional but would be helpful in counseling about active treatment.

Case 3: A 6-year-old experienced perioral urticaria and eye swelling developed when fed peanut butter at one year of age. The sIgE value at that time was 1.1 kU/L. There have been no other peanut exposures over the subsequent 5 years. The current SPT result is 4 mm greater than the negative control, and the sIgE value is 2.9 kU/L. Recommendation: the options at this stage are peanut component testing or BAT.

Case 4: A 4-year-old is being evaluated because the family is interested in OIT if the child is truly allergic to peanuts. A food panel test with a peanut IgE value of 0.9 kU/L was performed at one year of age because of eczema. There have been no peanut exposures. The current sIgE value is 1.9 kU/L. Although they are interested in OIT, the parents are very anxious about the possibility of an epinephrine-requiring reaction occurring during OFC. Recommendation: BAT testing highly correlates with OFC results and can substitute for OFC in this setting.

Case 5: A 6-month-old has had several episodes of facial erythema but no respiratory or gastrointestinal symptoms on peanut butter exposure. The SPT result is 9 mm. Recommendation: obtain a baseline sIgE value for future reference and low-dose open challenge to determine a tolerated dose that could be taken daily for 6–12 months, at which time the child could be reevaluated.

CONCLUSION

Understanding the strengths and limitations of diagnostic food allergy tests and using them in an organized manner is a crucial element of optimizing and customizing the care of patients with food allergy.

ACKNOWLEDGMENT

I thank Dr. Robert W. Sugerman for his helpful review of the manuscript.

REFERENCES

1. Peterson MC, Holbrook JH, Von Hales D, et al. Contributions of the history, physical examination, and laboratory investigation in making medical diagnoses. West J Med. 1992; 156:163–165.
2. Muraro A, de Silva D, Halken S, et al. Managing food allergy: GA^{2}LEN guideline 2022. World Allergy Organ J. 2022; 15:100687.
3. Anagnostou A, Lieberman J, Greenhawt M, et al. The future of food allergy: challenging existing paradigms of clinical practice. Allergy. 2023; 78:1847–1865.
4. Riggioni C, Ricci C, Moya B, et al. Systematic review and meta-analyses on the accuracy of diagnostic tests for IgE-mediated food allergy. Allergy. 2024; 79:324–352.
5. Ruethers T, Johnston EB, Karnaneedi S, et al. Commercial shellfish skin prick test extracts show critical variability in allergen repertoire. Allergy. 2023; 78:3261–3265.
6. Rosen JP, Selcow JE, Mendelson LM, et al. Skin testing with natural foods in patients suspected of having food allergies: is it a necessity? J Allergy Clin Immunol. 1994; 93:1068–1070.
7. Kleine-Tebbe J, Poulsen LK, Hamilton RG. Quality management in IgE-based allergy diagnostics. LaboratoriumsMedizin. 2016; 40:81–96.
8. Santos AF, Brough HA. Making the most of *in vitro* tests to diagnose food allergy. J Allergy Clin Immunol Pract. 2017; 5:237–248.
9. Keet C, Plesa M, Szelag D, et al. Ara h 2-specific IgE is superior to whole peanut extract-based serology or skin prick test for diagnosis of peanut allergy in infancy. J Allergy Clin Immunol. 2021; 147:977–983.e2.
10. Sonneveld LJH, Emons JAM, Arends NJT, et al. ALEX versus ISAC multiplex array in analyzing food allergy in atopic children. Clin Mol Allergy. 2022; 20:10.
11. Santos AF, Kulis MD, Sampson HA. Bringing the next generation of food allergy diagnostics into the clinic. J Allergy Clin Immunol Pract. 2022; 10:1–9.
12. Suprun M, Kearney P, Hayward C, et al. Predicting probability of tolerating discrete amounts of peanut protein in allergic children using epitope-specific IgE antibody profiling. Allergy. 2022; 77:3061–3069.
13. Santos AF, Alpan O, Hoffmann H-J. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice. Allergy. 2021; 76:2420–2432.
14. Kim T, Yu J, Li H, et al. Validation of inducible basophil biomarkers: time, temperature and transportation. Cytometry B Clin Cytom. 2021; 100:632–644.
15. Santos AF, Douiri A, Becares N, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol. 2014; 134:645–652.
16. Mukai K, Gaudenzio N, Gupta S, et al. Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. J Allergy Clin Immunol. 2017; 139:889–899.e11.
17. Dinakar C, Shroba J, Portnoy JM. The transforming power of proximity food challenges. Ann Allergy Asthma Immunol. 2016; 117:135–137. □