

Diagnosing Peanut Allergy with Fewer Oral Food Challenges



Jennifer J. Koplin, PhD^{a,b}, Kirsten P. Perrett, MD, PhD^{a,b,c}, and Hugh A. Sampson, MD^d Parkville, Victoria, Australia; and New York, NY

Diagnosis of peanut allergy presents a significant clinical challenge. Accurate diagnosis is critical for patient management and prevention of allergic reactions, whereas overdiagnosis or failure to diagnose tolerance in a previously allergic patient can lead to unnecessary dietary restrictions and impaired quality of life. Oral food challenges, the criterion standard for diagnosis, pose a risk of potentially severe allergic reactions, and are time- and resource- intensive. In this article, we review other currently available tests for peanut allergy and present the strengths and weaknesses of each to assist the clinician in determining which test might be appropriate for their patients, as well as highlighting emerging tests currently in development. Traditional tests for peanut-specific IgE (skin prick testing and specific IgE) remain useful as first-line tests—a negative test result is useful for excluding peanut allergy and a high positive result has a high specificity for peanut allergy. For those with an intermediate positive test result, Ara h 2 testing might be useful as a second step. Basophil activation tests and peanut protein epitope-specific IgE analyses appear promising in recent studies; however, further research is required into standardization, validation, and cost-effectiveness. Given the limitations of existing tests for peanut allergy, there remains a clear need for improvement. Finding a safe and affordable method for peanut allergy diagnosis that is both sensitive and specific remains an active area of research. © 2018 American

Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:375-80)

Key words: Peanut allergy; Diagnosis; Skin prick test; Specific IgE; Basophil activation test; Mast cell activation test; Histamine release assay

INTRODUCTION

Accurate diagnosis of peanut allergy, critical for patient management and prevention of allergic reactions, presents a significant clinical challenge. Oral food challenges (OFCs), particularly double-blind placebo-controlled OFCs, are the criterion standard for diagnosis of peanut as well as other food allergies. However, food challenges pose a risk of potentially severe allergic reactions, and are time- and resource- intensive.

Diagnosis of IgE-mediated peanut allergy may be straightforward in the context of a clear clinical history of reaction following known peanut exposure and evidence of peanut-specific IgE. However, for patients who present with an unclear history of peanut ingestion or reaction, or infants without a clear history of tolerating age-appropriate portions of peanut, but who are at high risk due to history of early severe eczema or egg allergy, an accurate diagnostic test is vital. Similarly, accurate diagnostic tests are needed to determine resolution of allergy (development of tolerance) in patients with a history of peanut allergy.

In this article, we review currently available tests for peanut allergy and present the strengths and weaknesses of each to assist the clinician in determining which test might be appropriate for their patients. We also highlight emerging tests currently in development.

DEFINITIONS

The ability of a diagnostic test to correctly classify individuals into 2 categories (allergic or not allergic) is assessed by 2 parameters, sensitivity and specificity.

Sensitivity is defined as the proportion of true positives (peanut-allergic individuals) correctly identified as peanut allergic by the diagnostic test. In other words, for a test with high sensitivity, very few truly allergic patients will have a false-negative test result.

Specificity is defined as the proportion of true negatives (peanut-tolerant individuals) correctly identified as peanut tolerant by the diagnostic test. In other words, for a test with high specificity, very few peanut-tolerant patients will have a false-positive test result.

Clinically, it is common to use positive and negative predictive values, which are more easily interpreted because they provide a

^aPopulation Allergy Research Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

^bSchool of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia

^cDepartments of Allergy and Immunology and General Medicine, Royal Children's Hospital, Parkville, Victoria, Australia

^dDepartment of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY K.P.P. is supported by a Melbourne Children's Clinician-Scientist Fellowship. Research at the Murdoch Children's Research Institute is supported by the Victorian Government's Operational Infrastructure Program.

Conflicts of interest: H. A. Sampson reports being a part-time employee of DBV Technologies; receiving consultant fees from N-Fold, LLC, and UCB SA, and royalties from UpToDate and Elsevier; holding stock options in DBV Technologies and N-FOLD; and receiving grants to his institution from the National Institutes of Health, National Institute of Allergy and Infectious Diseases, and Food Allergy Research & Education. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication October 1, 2018; revised November 11, 2018; accepted for publication November 12, 2018.

Available online December 21, 2018.

Corresponding author: Hugh A. Sampson, MD, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, New York, NY 10029. E-mail: hugh.sampson@mssm.edu.

2213-2198

© 2018 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2018.11.010>

Abbreviations used

BAT- basophil activation test

HR- histamine release

MAT- mast cell activation test

OFC- oral food challenge

PPV- positive predictive value

sIgE- specific IgE

SPT- skin prick test

measure of the probability that a patient is truly allergic or tolerant, respectively.

Positive predictive value (PPV) is defined as the proportion of test-positive patients who are truly peanut allergic.

Negative predictive value is defined as the proportion of test-negative patients who are truly peanut tolerant.

Positive and negative predictive values are related to the sensitivity and specificity of the diagnostic test; however, they are also dependent on the underlying prevalence of disease. For example, if a test with a particular fixed specificity and sensitivity threshold is performed in a patient presenting to an allergy clinic with a clear history of reactions to peanut (likely truly peanut allergic), the PPV for a given test will be higher compared with the PPV for the same test applied in a community setting where few individuals will be peanut allergic. Keet et al¹ provide a clear example of this issue in an article that shows that PPVs for specific IgE (sIgE) derived from clinic populations cannot be meaningfully applied to the general population.

It has been proposed that likelihood ratios, which compare the probability of a positive skin prick test (SPT) result in patients with and without food allergy, should be the measure of choice because they have the potential to be independent of disease prevalence and could therefore be applied across different allergy settings.² The calculation and appropriate application of likelihood ratios does however require several steps, which may explain why this procedure has so far received limited use in clinical practice.

A NOTE ABOUT STUDY DESIGNS

Many of the early studies investigating the accuracy of diagnostic tests such as SPTs and measurement of peanut sIgE in serum used data from patients presenting to allergy clinics for peanut OFC. Several issues need to be considered when interpreting the results of these studies: OFCs are typically offered clinically to only those patients deemed by the allergist to be at low risk of reacting (eg, because of recent history of accidental exposure without reaction); patients seen in allergy clinics are likely to have a more severe profile of allergic disease compared with patients seen by a general practitioner (eg, multiple food allergies or more severe reactions to foods); and food challenge stopping criteria are often not standardized (eg, some challenges may be called positive if a patient develops subjective symptoms, whereas others are stopped only for objective symptoms). As a result, the findings of these studies might not be generalizable to the wider group of all patients with suspected food allergy, and results may vary even between different tertiary allergy centers due to differences in patient populations. Finally, most studies to date have been performed in pediatric populations with limited studies in adults, and the relationship between SPT wheal

sizes and sIgE levels to peanut and true peanut allergy may vary by age.

SKIN PRICK TESTING

In Australia, most pediatric allergy clinics use SPTs in preference to sIgE testing because results are immediately available. SPT has consistently been shown to have high sensitivity; in other words, peanut allergy is highly unlikely in an individual with a negative SPT result (usually defined as a wheal size of <3 mm). In a systematic review and meta-analysis, Soares-Weiser et al³ calculated a pooled sensitivity of 94.7% (95% CI, 87.9-97.8) for an SPT with a wheal size of greater than or equal to 3 mm using data from 5 studies with a combined total of 499 participants and 245 cases of peanut allergy.

Conversely, a positive SPT result has a low specificity. In the same systematic review, the pooled specificity for an SPT with a wheal size of greater than or equal to 3 mm was only 61.0% (95% CI, 46.6-73.6). Consequently, several studies have attempted to calculate SPT thresholds above which an individual has a 95% probability of being clinically allergic (95% PPV), to determine when an OFC is likely to be unnecessary. Using data from the *HealthNuts* study in Australia, Peters et al⁴ calculated PPVs for peanut allergy in a population-recruited cohort of 5276 infants who were 1 year old. All infants underwent SPT to peanut, and those with any detectable wheal (wheal size $\geq$ 1 mm) underwent an OFC irrespective of their SPT wheal size ($n = 435$, of which 148 were positive on challenge), as did 200 randomly selected SPT negative controls. An SPT wheal size of 8 mm had a 95% PPV for peanut allergy, a specificity of 98% (95% CI, 95-99), and a sensitivity of 54% (95% CI, 46-62).

In a follow-up of the same peanut-allergic individuals at age 4 years, Peters et al⁵ reported that a decreasing wheal size between age 1 and 4 years predicted development of tolerance to peanut. Of 156 peanut-allergic infants, 22% were tolerant to peanut on repeat food challenge at age 4 years. On average, SPT responses decreased by 4 mm in children whose peanut allergy had resolved by age 4 years, compared with an average increase of 3 mm in children with persistent peanut allergy. However, there was significant variability between individuals, with some children developing tolerance despite a lack of decrease in SPT wheal size, and others remaining allergic despite a significant decrease. At age 4 years, an SPT wheal size of 8 mm or greater had a 95% PPV for persistent peanut allergy. Similar observations were reported previously in a clinical cohort of peanut-allergic children of a similar age.⁶

A key advantage of SPT is that results are immediately available. The high sensitivity makes this a useful tool to exclude peanut allergy in an individual with an unclear clinical history if the test result is negative. Although a positive SPT result has poor specificity overall, use of a higher cutoff can improve specificity and several studies conducted in different populations have confirmed that an 8-mm threshold provides a high specificity and PPV for peanut allergy. However, individuals with a smaller SPT wheal size are still likely to require OFC or further testing to determine their food allergy status in the absence of a clear clinical history. Various factors have also been shown to influence SPT wheal size, including choice of SPT device and reagents, technique, and even site of SPT,⁷ and these need to be taken into account when interpreting the results of the above studies and applying these in different clinical settings.

PEANUT sIgE (WHOLE PEANUT)

Measurement of serum peanut sIgE levels is common as a first-line test for peanut sensitization in US pediatric allergy clinics. Like SPT, sIgE measurement has a high sensitivity but a relatively low specificity. In a systematic review and meta-analysis, Soares-Weiser et al³ calculated a pooled sensitivity of 96.3% (95% CI, 91.6-98.4) and a specificity of 59.3% (95% CI, 45.4-72.0) for peanut sIgE (mixed cutoffs) using data from 5 studies with a combined total of 817 participants and 452 cases of peanut allergy. Similar to SPT, decreasing sIgE levels to peanut are associated with resolution of peanut allergy.⁵

Studies have also attempted to determine a 95% PPV for sIgE testing, with varying results. In the *HealthNuts* study, sIgE levels above 34 kU_A/L had a 95% PPV for peanut allergy at age 1 year, whereas at age 4 years the corresponding 95% PPV was 2.1 kU_A/L. Other studies have suggested a threshold of 15 kU_A/L for children with a median age of 3.8 years.^{8,9} These differences are likely to be attributed to differences in study populations and test methods. As we describe above, the PPV for a given sIgE threshold is expected to be higher if the test is performed in patients presenting to an allergy clinic with a history of peanut reactions (high likelihood of being truly peanut allergic) compared with a community setting where few individuals will be peanut allergic.¹ Peters et al⁴ reported that the sIgE threshold with 95% PPV for peanut allergy was significantly lower in infants with a history of reacting to peanut (1.0 kU_A/L) compared with those without a history of reaction (39 kU_A/L).

Measuring sIgE levels has advantages over SPT in some settings because it can be performed even in patients with severe eczema who may not have a patch of clear skin for SPT, and does not require patients to avoid antihistamines before testing. General practitioners without training and experience in performing and interpreting SPT may also be more comfortable with ordering sIgE testing. However, cutoffs that define a very high likelihood of peanut allergy appear to be more variable between studies.

sIgE TO PEANUT COMPONENTS

In the past 5 to 10 years, more studies have focused on measuring IgE to specific peanut components (component-resolved diagnosis) rather than to whole peanut in an attempt to improve on the high rate of false-positive results for SPT and sIgE levels. These test are now commercially available and in widespread clinical use.

Peanut components that have been tested in clinical studies include Ara h 1, 2, 3, 6, 8, and 9. A systematic review by Kleman et al¹⁰ of 22 studies, 21 in pediatric populations, concluded that Ara h 2 provided the best combination of diagnostic accuracy measures, and was mainly superior to SPT and sIgE for diagnosing peanut allergy in case of a positive test result. Ara h 2 testing may be useful as a second screening step in patients with a positive SPT result or whole peanut sIgE result to reduce the requirement for OFCs.¹¹ Similar to whole peanut sIgE, increasing levels of component sIgE to Ara h 2 are also associated with an increasing likelihood of true peanut allergy.¹¹ A more recent systematic review that included only 3 studies with double-blind placebo-controlled OFC outcomes suggested that Ara h 6 might have a higher diagnostic accuracy for peanut allergy compared with Ara h 2, but comparison between studies was difficult due to varied cutoff values, and concluded that more

research is required to determine clinically appropriate cutoff values and cost-effectiveness of component-resolved diagnosis approaches for peanut allergy.¹² A final issue to consider is that patterns of sensitization to peanut components may vary in different regions because of the effects of sensitization to cross-reacting pollens.

A proposed diagnostic approach combining SPT or sIgE measurements with Ara h 2 testing is highlighted in Figure 1. Using this 2-step approach, when managing children with a clinical history of an IgE-mediated reaction to peanut in the middle range of sIgE or SPT sensitization who may not have previously been challenged, if they have a low Ara h 2 result, clinicians may now opt to perform an oral peanut challenge.

BASOPHIL ACTIVATION TEST

Basophils, like mast cells, express a high-affinity IgE receptor. Cross-linking of IgE bound to FcεRI receptors on the cell surface results in rapid release of granule-stored inflammatory mediators (eg, histamine) and upregulation of cell surface receptors such as CD63 and CD203c. The basophil activation test (BAT) is an *in vitro* functional assay that assesses the expression of these activation markers on the surface of live basophils in whole fresh blood by flow cytometry following stimulation with peanut allergen.¹³ In a key study of peanut-allergic patients, BAT showed a dose-dependent expression of activation markers CD63 or CD203c. Conversely, sensitized tolerant and nonsensitized nonallergic patients either did not express or showed a much lower expression of these activation markers following stimulation with peanut allergen.¹⁴

The key strength of the BAT is the ability to accurately differentiate between allergic and tolerant patients and have a superior specificity and comparable sensitivity compared with other diagnostic tests. In this pivotal study, BAT was shown to have a 95% PPV and 98% negative predictive value, and allowed a two-thirds reduction in the number of OFCs required.¹⁴ However, it should be noted that the population prevalence of peanut allergy in this allergic cohort was approximately 40% so PPV will be higher than when applied to a more general population. When the same authors validated the BAT in clinical practice using it selectively in various multistep strategies following an equivocal test (SPT, sIgE, or Ara h 2), the most accurate and cost-effective analysis was found to be when an equivocal peanut SPT or Ara h 2-sIgE was followed by BAT. In this way, the number of OFCs required was reduced significantly (a reduction of 97%). It has been proposed that following this diagnostic pathway for selected cases where the history, SPT, and/or IgE are not definitive for the diagnosis of food allergy, a BAT should be performed. A positive BAT result would remove the need for an OFC; however, a patient with a negative BAT result or nonresponder basophils (basophils that do not respond to IgE-mediated stimulants) would still require the criterion standard OFC test to confirm or refute the diagnosis. It has been suggested that this occurs in approximately 10% to 15% of individuals.¹⁵

The utility of using BAT to diagnose peanut allergy has been assessed in multiple studies in recent years with overall high sensitivity and specificity with ranges from 83% to 92% and 77% to 100%, respectively.^{14,16-18}

The BAT also has some other clear strengths: being able to be performed even in children with extensive eczema, or in those

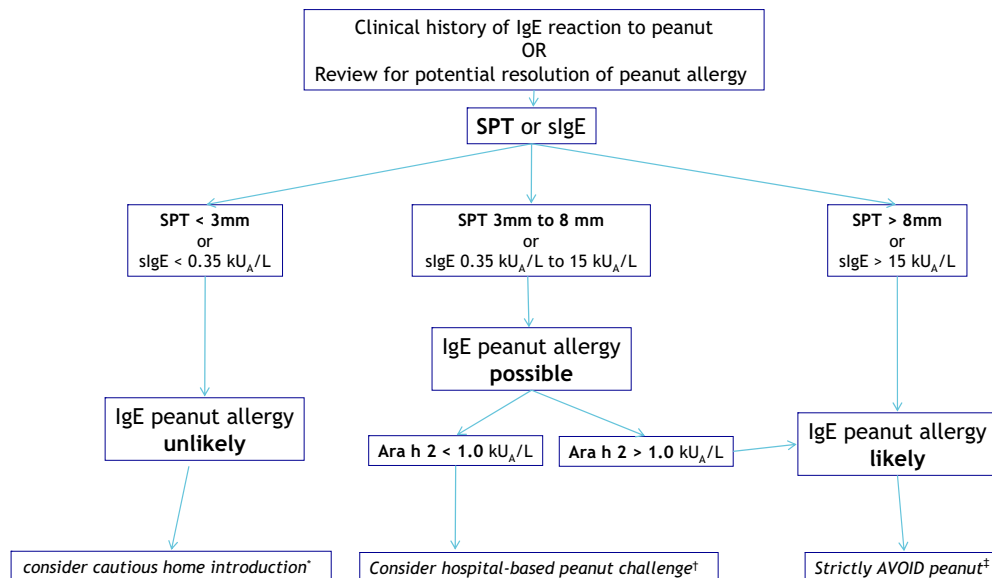


FIGURE 1. Suggested algorithm for the diagnosis of peanut allergy in children with a history suggestive of an IgE-mediated reaction to peanut or review for potential resolution of peanut allergy. *In infants and young children, an SPT wheal of 2 mm may indicate clinical allergy and Ara h 2 may be indicated to determine whether a hospital peanut challenge should be considered. Cautious home introduction should be done only under allergist guidance and may not be recommended where there is a history of severe anaphylaxis. †Future diagnostic tests (eg, Ara h 6, BAT, or epitope sIgE analyses) may assist in reducing the number of children requiring hospital OFCs for diagnosis. ‡Depending on child's age, SPT and/or sIgE should be repeated at regular intervals in children avoiding peanut to determine need for ongoing avoidance or possible development of tolerance. A decrease in SPT wheal size or sIgE may indicate an increased likelihood that peanut allergy has resolved. For children with whole peanut IgE level of more than 15 kU_A/L, Ara h2 testing may be helpful in some infants and children without a history of reaction because some studies have demonstrated higher 95% PPV cutoffs in infants and children without a history of reaction.⁴

who have had recent antihistamine medication and a high safety profile (as they are performed *in vitro* not *in vivo*). However, the major weakness of the BAT is that fresh whole blood (<4 hours from sampling) is required although only a small volume of approximately 1 mL is needed. In addition, the laboratory method and analysis are not standardized. The method can vary with regard to sample anticoagulation, reagents, allergen extracts used, flow cytometry equipment, settings, and criteria for analyses.

Although the BAT appears to be an extremely promising test, offering higher specificity and safety for the patient, future research into standardization and validation of the BAT, the impact of BAT on health outcomes, and cost-effectiveness are urgently needed.

MAST CELL ACTIVATION TEST

Mast cells have traditionally been considered the main effector cells in patients with allergic reactions (activated through IgE cross-linking of FcεRI on the cell surface, resulting in *de novo* synthesis and release of inflammatory mediators) and it therefore makes sense that a test to measure their activation might make a good *in vitro* diagnostic test. Recently, this novel test has been validated against existing tools.^{15,19} In the mast cell activation test (MAT), the function of the individual's allergen sIgE antibodies' ability to elicit mast cell degranulation is assessed. Initially human blood-derived mast cells need to be generated from peripheral blood precursors from healthy donors—a technique that requires time and specific expertise.¹⁹ Once mast cell

cultures are established, they are sensitized passively with individual patient sera, incubated with allergen *in vitro*, and assessed for mast cell activation by flow cytometry.

A definite advantage of the MAT over the BAT is the use of stored plasma rather than fresh whole blood, which allows testing of samples that may have been collected far from the laboratory or previously stored. MATs like BATs have demonstrated high specificity when used to diagnose peanut allergy.^{15,19} Santos et al¹⁵ felt that the BAT showed greater diagnostic accuracy compared with their MAT assay (using LAD2 mast cell line) because of better sensitivity; however, the MAT provided definitive results for all individuals with non-responding basophils. Conversely, Bahri et al¹⁹ felt that their MAT assay (which used a different cell line, CD117⁺CD34⁺) was superior to BAT and all other available diagnostic tests (area under the curve, 0.99). Although there is certainly some further work to be done to standardize and validate the MAT assay, it is clear that it too is a promising novel diagnostic tool for the diagnosis of peanut allergy.

HISTAMINE RELEASE ASSAYS

Another new *in vitro* assay, the histamine release (HR) assay, measures the amount of histamine released from activated basophils. In this version of the basophil diagnostic test, whole blood (collected < 24 hours from sampling) is incubated with peanut extract or anti-IgE, released histamine binds to a solid glass fiber phase, and unbound blood is removed with repeated washing. Trapped histamine is obtained from the solid phase

by increasing pH and quantified flurometrically. A modified passive HR assay using an individual's serum has also been developed to overcome the need for fresh blood.²⁰ Recently, these 2 *in vitro* basophil tests (BAT, HR assay, and passive HR assay) were compared. The BAT displayed a significantly higher CD sensitivity (a measure of basophil allergen sensitivity) compared with HR assay and passive HR assay (80%, 23%, 11%)²⁰; however, excluding inconclusive tests, diagnostic accuracy was comparable. Because of the strengths of using stored sera, the authors suggest that the passive HR assay might have clinical use for confirming peanut allergy, but because it incorrectly identified subjects as nonallergic, it could not be used to exclude it.

OTHER EMERGING DIAGNOSTIC TESTS

Given the limitations of existing tests for peanut allergy, there remains a clear need for improvement. Finding a safe and affordable method for peanut allergy diagnosis that is both sensitive and specific remains an active area of research. Two decades ago it was shown that IgE antibodies to sequential (linear) epitopes of food proteins correlated closely with reactivity to specific foods.²¹ Subsequently, IgE to allergenic (IgE-binding) epitopes were evaluated using short sequential peptides of 15 to 20 amino acids bound to a solid phase (eg, initially cellulose-derived "spot membranes" and later microarrays) using immunofluorescence. Beyer et al²² identified 5 immunodominant epitopes in selected peanut allergen peptides in 2003 that more accurately predicted persistent peanut allergy. Several years later, using microarrays containing peptides of the major peanut allergens, Ara h 1, 2, and 3, investigators identified specific epitopes bound more by the IgE antibodies of peanut-allergic patients compared with IgE of peanut-sensitized, tolerant patients, which also correlated with severity of allergic reactions.^{23,24} Given the heterogeneity of IgE- and IgG₄-binding profiles among peanut-allergic patients, machine-learning methods have been enlisted, which markedly enhance the diagnostic accuracy and utility of the microarray.²⁵ Most recently, a Luminex-based peptide assay has been developed that requires lower serum volumes and has lower interassay variability, increased sensitivity, and ease of automation compared with the microarray.²⁶ If current studies are validated, the Luminex-based peptide assay will provide greater accuracy in diagnosing clinical peanut allergy, identify those patients who will outgrow their peanut allergy, provide information on potential severity of future reactions, and predict who will respond well to immunotherapy.²⁶ Other studies looking at peanut-stimulated T-cell cytokine production and mRNA expression profiles may also prove valuable as future diagnostic tests of peanut allergy.

CONCLUSIONS

Forty-eight years ago the double-blind placebo-controlled food challenge was shown to be the "criterion standard" for diagnosing food allergy. Over the past 4 decades diagnostic testing has progressed from the SPT to crude measurements of serum IgE to whole peanut protein (radioallergosorbent test) to quantitative measurement of IgE to whole peanut protein, component proteins comprising peanut, and most recently to allergenic epitopes of Ara h 1, 2, 3, and 6. With this evolution, the clinician can now provide patients with more accurate

diagnoses, but with new tests that may soon be available for clinical use (BAT and epitope analysis), the clinician may be able to provide patients with information about the degree and natural history of their peanut allergy.

REFERENCES

1. Keet CA, Wood RA, Matsui EC. Limitations of reliance on specific IgE for epidemiologic surveillance of food allergy. *J Allergy Clin Immunol* 2012;130:1207-1209.e10.
2. Akobeng AK. Understanding diagnostic tests 2: likelihood ratios, pre- and post-test probabilities and their use in clinical practice. *Acta Paediatr* 2007;96:487-91.
3. Soares-Weiser K, Takwoingi Y, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. The diagnosis of food allergy: a systematic review and meta-analysis. *Allergy* 2014;69:76-86.
4. Peters RL, Allen KJ, Dharmage SC, Tang MLK, Koplin JJ, Ponsonby A-L, et al. Skin prick test responses and allergen-specific IgE levels as predictors of peanut, egg, and sesame allergy in infants. *J Allergy Clin Immunol* 2013;132:874-80.
5. Peters RL, Allen KJ, Dharmage SC, Koplin JJ, Dang T, Tilbrook KP, et al. Natural history of peanut allergy and predictors of resolution in the first 4 years of life: a population-based assessment. *J Allergy Clin Immunol* 2015;135:1257-62.
6. Ho MHK, Wong WHS, Heine RG, Hosking CS, Hill DJ, Allen KJ. Early clinical predictors of remission of peanut allergy in children. *J Allergy Clin Immunol* 2008;121:731-6.
7. Werther RL, Choo S, Lee KJ, Poole D, Allen KJ, Tang ML. Variability in skin prick test results performed by multiple operators depends on the device used. *World Allergy Organ J* 2012;5:200-4.
8. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
9. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891-6.
10. Klemans RJB, van Os-Medendorp H, Blankestijn M, Bruijnzeel-Koomen CAFM, Knol EF, Knulst AC. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy* 2015;45:720-30.
11. Dang TD, Tang M, Choo S, Licciardi PV, Koplin JJ, Martin PE, et al. Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. *J Allergy Clin Immunol* 2012;129:1056-63.
12. Flores Kim J, McCleary N, Nwaru BI, Stoddart A, Sheikh A. Diagnostic accuracy, risk assessment, and cost-effectiveness of component-resolved diagnostics for food allergy: a systematic review. *Allergy* 2018;73:1609-21.
13. Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008;74B:201-10.
14. Santos AF, Douiri A, Bécarea N, Wu S-Y, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol* 2014;134:645-52.
15. Santos AF, Couto-Francisco N, Bécarea N, Kwok M, Bahnson HT, Lack G. A novel human mast cell activation test for peanut allergy. *J Allergy Clin Immunol* 2018;142:689-691.e9.
16. Glaumann S, Nopp A, Johansson SGO, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy* 2012;67:242-7.
17. Javaloyes G, Goikoetxea MJ, Garcia Nunez I, Sanz ML, Blanca M, Scheurer S, et al. Performance of different *in vitro* techniques in the molecular diagnosis of peanut allergy. *J Investig Allergol Clin Immunol* 2012;22:508-13.
18. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F, et al. Basophil activation tests for the diagnosis of food allergy in children. *Clin Exp Allergy* 2009;39:1234-45.
19. Bahri R, Custovic A, Korosec P, Tsoumani M, Barron M, Wu J, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. *J Allergy Clin Immunol* 2018;142:485-496.e16.
20. Larsen LF, Juel-Berg N, Hansen KS, Clare Mills EN, Ree R, Poulsen LK, et al. A comparative study on basophil activation test, histamine release assay, and passive sensitization histamine release assay in the diagnosis of peanut allergy. *Allergy* 2017;73:137-44.

21. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997;159:2026-32.
22. Beyer K, Ellman-Grunther L, Jarvinen KM, Wood RA, Hourihane J, Sampson HA. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol* 2003;112:202-7.
23. Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004;113:776-82.
24. Flinterman AE, Knol EF, Lencer DA, Bardina L, den Hartog Jager CF, Lin J, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol* 2008;121:737-743. e10.
25. Lin J, Bruni FM, Fu Z, Maloney J, Bardina L, Boner AL, et al. A bioinformatics approach to identify patients with symptomatic peanut allergy using peptide microarray immunoassay. *J Allergy Clin Immunol* 2012;129:1321-1328. e5.
26. Sackesen C, Suarez-Farinas M, Sillva R, Lin J, Schmidt S, Getts R, et al. A new luminex-based peptide assay to identify reactivity to baked, fermented and whole milk [published online ahead of print July 29, 2018]. *Allergy*. <https://doi.org/10.1111/all.13581>.