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ORIGINAL ARTICLE

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Accurate and reproducible diagnosis of peanut allergy using epitope mapping

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Abstract

Background: Accurate diagnosis of peanut allergy is a significant clinical challenge. Here, a novel diagnostic blood test using the peanut bead-based epitope assay ("peanut BBEA") was developed utilizing the LEAP cohort and then validated using two independent cohorts.

Methods: The development of the peanut BBEA diagnostic test followed the National Academy of Medicine's established guidelines with discovery performed on 133 subjects from the non-interventional arm of the LEAP trial and an independent validation performed on 82 subjects from the CoFAR2 and 84 subjects from the POISED study. All samples were analyzed using the peanut BBEA methodology, which measures levels of IgE to two Ara h 2 sequential (linear) epitopes and compares their combination to a threshold pre-specified in the model development phase. When a patient has an inconclusive outcome by skin prick testing (or sIgE), IgE antibody levels to this combination of two epitopes can distinguish whether the patient is "Allergic" or "Not Allergic." Diagnoses of peanut allergy in all subjects were confirmed by double-blind placebo-controlled food challenge and subjects' ages were 7–55 years.

Results: In the validation using CoFAR2 and POISED cohorts, the peanut BBEA diagnostic test correctly diagnosed 93% of the subjects, with a sensitivity of 92%, specificity of 94%, a positive predictive value of 91%, and negative predictive value of 95%.

Conclusions: In validation of the peanut BBEA diagnostic test, the overall accuracy was found to be superior to existing diagnostic tests for peanut allergy including skin prick testing, peanut sIgE, and peanut component sIgE testing.

KEYWORDS
BBEA, diagnosis, epitopes, IgE, peanut

1 | INTRODUCTION

The prevalence of peanut allergy among children in the United States is estimated to be ~2%.[1] The gold standard for diagnosis of peanut allergy is the double-blind placebo-controlled food challenge (DBPCFC), however, the DBPCFC is time- and resource-intensive, not widely available in clinical practice, and a potentially risky procedure.[2] More commonly, diagnosis is determined using a combination of patient history, skin prick tests (SPT), and peanut-specific IgE (sIgE) and peanut allergen component-specific testing (eg, Ara h 2).[3-6] Heuristic methods for optimally using these tools, on their own or in combination, have been explored.[3,4] Nevertheless, the diagnostic performance of these tools has fallen short both in terms of accuracy and level of evidence. In particular, it is estimated that the rate of peanut allergy "over-diagnosis" may exceed 60%.[7] More recently, the basophil activation test (BAT) has been assessed demonstrating higher accuracy than the SPT and Ara h 2 testing (used independently), but statistical superiority was not established.[8] Additionally, BAT testing has been associated with a 5–10% non-responsive rate to IgE-mediated stimulation[9] and requires fresh whole blood.

An ideal alternative diagnostic test for the oral food challenge would have the following attributes:

- Be relatively non-invasive, such as a blood-based test.
- Be rigorously developed and documented in accordance with national guidelines such as those established by the National Academy of Medicine.[10]
- Be clinically validated on multiple independent and well-characterized cohorts.
- Be clinically validated on subjects whose allergy status has been largely confirmed by DBPCFC.
- Be clinically and analytically validated in a laboratory with documented standard operating procedures, and ideally, certified and accredited by an external regulatory group such as Clinical Laboratory Improvement Amendments (CLIA) or College of American Pathology.
- Has a diagnostic performance that is at least 90% accurate as compared to DBPCFC; and superior to established clinical tests for diagnosis.

These criteria ensure that an alternative diagnostic test is accurate with both a high level of clinical and analytical validation.

Here, we present the development and clinical validation of a test for the diagnosis of peanut allergy that meets the criteria above. The diagnostic test is based on the peanut Bead-Based Epitope Assay (peanut BBEA), a plasma-based test that utilizes the previously characterized BBEA assay for measuring IgE antibody binding to sequential (linear) peanut epitopes.[11,12] The diagnostic test, herein referred to as *peanut BBEA diagnostic test*, utilizes the level of two highly informative "sequential epitope-specific IgE" antibodies (ses-IgE) to predict the outcome of the oral food challenge to peanut. The peanut BBEA diagnostic test was validated in two independent clinical cohorts, of which both demonstrated sensitivity and specificity above 90% in comparison with DBPCFC, and accuracy significantly and statistically superior to established diagnostic tests such as the SPT, and whole peanut and peanut component sIgE testing. Of note, although we have chosen to use the terms peptide and epitope interchangeably, these preselected overlapping peptides may not represent the actual peanut epitope's exact amino acid sequence, as evidenced by IgE binding to 2 or more contiguous overlapping peptides.

2 | METHODS

2.1 | Ses-IgE quantification using peanut BBEA

For all three cohorts, subjects were randomized across 96-well plates and assayed in triplicates [13] using BBEA as previously described.[11] Briefly, sixty-four clinically relevant (informative) sequential peptides belonging to Ara h 1 (n = 34), Ara h 2 (n = 16), and Ara h 3 (n = 14) were synthesized (CS Bio), coupled to LumAvidin beads (Luminex Corporation) and stored in PBS-TBN buffer (1xPBS + 0.02%Tween20 + 0.1%BSA). A master mix of peptide-coupled beads was made in PBS-TBN buffer and 100 μL of the bead master mix was added to 96-well filter plates (1000 beads/well). After washing the beads, 100 μL of the subject's plasma at 1:10 dilution was added to the wells. The plates were incubated on a shaker for 2 hours at 300 rpm at room temperature. Excess plasma was then removed, and the plate was washed. 50 μL/well of mouse anti-human phycoerythrin (PE) conjugated IgE-PE (Thermo-Pierce Antibodies, Clone BE5, diluted 1:50 in PBS-TBN), secondary antibody was added, and plates were incubated for 30 minutes. After a final wash, 100 μL of PBS-TBN buffer was added to each well to resuspend the beads, which were then transferred to fixed-bottom 96-well reading plates and quantified as median fluorescence intensity (MFI) on the Luminex 200 instrument (Luminex® 100/200™ System, Luminex Corporation) using default laser settings (635 nm for red laser and 532 nm for the green). The CoFAR2 cohort was assayed using an initial version of the assay including only 50 epitopes. This library was subsequently extended to 64 epitopes for the LEAP and POISED cohorts. However, the most informative epitopes, for example, Ara h 2_008 and Ara h 2_019, were present in both the 50- and 64-plex libraries.

Raw MFI data were $\log_2$-normalized, backgrounds subtracted, triplicates combined into a single value using the median function, and plates were normalized using a plate standard sample.[11] The lower limit of detection (LLOD) was determined using the CLSI (Clinical and Laboratory Standards Institute) EP34 guidelines[14] for determining and validating a LLOD. The methodology prescribed and followed utilized a dilution scheme to determine and validate the LLOD. This LLOD is then used to determine when an epitope is detectable at a reliable level above background.

2.2 | Luminex bead titration

Two plasma samples were prepared as follows: one representing a high peanut-specific IgE level (133.4 kU_A/L), high positive control, and a second representing a low peanut-specific IgE level (1.26 kU_A/L), low positive control. The BBEA assay was performed as described above[11] with one exception, epitope (peptide) coupled Luminex beads were titrated in the assay, 100 beads/well, 300 beads/well, 1000 beads/well, and 3000 beads/well.

2.3 | Discovery phase

The development of the peanut BBEA diagnostic test was based on 133 subjects (31 allergic, 102 non-allergic) randomly selected from subjects in the avoidance arm of the LEAP study (NCT00329784) where all diagnoses were confirmed by DBPCFC.[15] Plasma samples were obtained at baseline (4–11 months of age) and at approximately 2 and 5 years of age for each subject. Sequential epitope reactivity levels were measured for 64 epitopes using BBEA.

The two best diagnostic epitopes were determined at year 5 by exhaustive search over all epitope pairs and combined into a minimum detectability model where the lower limit of detection for each epitope was determined. Linear logistic regression was used to combine pairs of epitopes into models. The optimal model was then assessed using the plasma samples from year 2 to ensure consistency. The decision threshold that maximized accuracy was determined and documented (fully locked-down prior to validation).

We first de-identified the best pair of detectable epitopes whose reactivity levels at the time of the OFC could predict the 5-year OFC outcome. Using logistic regression with logit link function, we conducted an exhaustive search over all detectable epitope pairs within the same (Ara h 1, Ara h 2, and Ara h 3) allergens, selecting the pair that maximized the AUC.

The optimal model was then assessed using the plasma samples from year 2 to ensure consistency, obtaining almost identical results. The decision threshold, that is, the threshold such that patients above that level of reactivity are defined as allergic, was determined to maximize accuracy and properly documented (fully locked-down) prior to validation as $\left(\text{Ara h2\_008} + \frac{\text{Ara h2\_019}}{20}\right) \leq 0.20$.

2.4 | Validation

Validation of the locked-down algorithm for the peanut BBEA diagnostic test was performed on two independent cohorts of subjects from CoFAR2 (NCT00356174)[16,17] and POISED (NCT02103270).[18] In both studies, all subjects evaluated had their peanut allergy status confirmed by DBPCFC.

For the CoFAR2 cohort, plasma samples were obtained for all subjects in the study that underwent a DBPCFC by the year 5 visit (82 subjects, 23 allergic, 59 non-allergic), mostly at about 2 and 5 years of age. For validation of the peanut BBEA diagnostic test, only the samples obtained at the time of DBPCFC were used for validation. The year 2 samples were used to validate the selection of the 2 most informative epitopes.

For the POISED cohort, plasma samples were obtained from 42 randomly selected peanut-allergic subjects enrolled in the POISED study and 42 age-matched non-allergic subjects collected under a separate IRB approved protocol. POISED subjects had ages ranging from 7 to 55 years. All samples from CoFAR2 and POISED cohorts were analyzed using the BBEA assay to obtain the ses-IgE reactivity levels. The study was approved by the local Institutional Review Boards. All the study subjects provided informed consent.

2.5 | Performance evaluation

Performance of the peanut BBEA was determined by constructing the confusion matrix of DBPCFC versus peanut BBEA-based allergy diagnosis and then deriving sensitivity and specificity, as well as other diagnostic performance metrics. The confusion matrix is a 2 × 2 matrix with a tally of the true positives, false positives, false negatives, and true negatives. Data analyses were performed using Matlab R2019b and R version 4.0.2.

2.6 | Sequences of dominant epitopes

The sequences of all peptides have been published previously.[12,19] The two epitopes selected for the diagnostic model were Ara h2_008—WELQGDRRCQSQLER and Ara h2_019—DSYERDPYSPSQDPY, which belong to the Ara h 2.0101 allergen (GenBank ID ACN62248.1).

3 | RESULTS

The development of the peanut BBEA diagnostic test was based on a subset of the per protocol cohort of the LEAP study (NCT00329784) where all diagnoses were confirmed by DBPCFC [15] and ses-IgE profiles were obtained as previously reported.[19]

Validation of the locked-down algorithm was performed on two independent cohorts of subjects from CoFAR2 (NCT00356174)[16,17] and POISED (NCT02103270)[18] studies.

The demographic profiles of the three cohorts (LEAP, CoFAR2, and POISED) are presented in Table 1. Note that the LEAP cohort was used for the discovery of the epitope-based rule, whereas CoFAR2 and POISED were used to validate the algorithm derived from the LEAP cohort. The CoFAR2 validation cohort covered ages from 5 – 9 years whereas the POISED cohort covered the age group from 7 to 55 years of age. The diagnosis of peanut allergy was confirmed by DBPCFC. For LEAP and CoFAR2, DBPCFCs were performed at about 5 years of age.

TABLE 1 Demographic profiles of the three cohorts participating in the discovery and validation of the peanut BBEA diagnostic test

	LEAP		CoFAR2		POISED	
	Allergic	Non-Allergic	Allergic	Non-Allergic	Allergic	Non-Allergic
n	31	102	23	59	42	42
Enrollment Age (years)	0.66 (0.37–0.91)	0.68 (0.40–0.91)	0.87 (0.37–1.23)	0.81 (0.3–1.25)	11 (7–49)	10 (7–55)
Peanut sIgE (kU_A/L)	0.39 (0.01–79.50)	0.04 (0.01–87.70)	1.46 (0.00–24.45)	0.53 (0.00–60.32)	37.4 (0.41–869)	N/A

Note: Each cell contains the median and range of values. Ages for LEAP and CoFAR2 are reported for subjects at the time of enrollment into the longitudinal studies. Non-allergic controls for the POISED cohort did not have peanut-specific IgE (sIgE) performed.

3.1 | Peanut BBEA diagnostic test: Discovery

Two starting principles drove the derivation of the algorithm for the peanut BBEA diagnostic test:

- SPT wheal diameter and sIgE antibody levels are effective for ruling-in and ruling-out peanut allergy at high and low values, respectively.
- IgE-mediated allergic reactions require two epitopes from the same peanut allergen to crosslink IgE.

Previous work has established that sufficiently high and low values for the SPT wheal diameter or sIgE level are needed to rule-in and rule-out peanut allergy.[3,4] Naturally, those tests are broadly used in clinical practice. Consequently, to create a decision rule for subjects with non-extreme sIgE/SPT values, we first identified a pair of epitopes from the same peanut allergen (Ara h 1, Ara h 2, or Ara h 3) that were most highly associated with clinical allergy. Furthermore, it was required that these epitopes were reliably detected in all peanut-allergic subjects. Utilizing plasma samples obtained at 5 years of age from the LEAP cohort, where results of DBPCFC were available, the epitope pair from the same peanut allergen with the highest area under the curve (AUC) and reliably detected were Ara h 2_008 and Ara h 2_019 (AUC = 74%; Figure 1A). This observation was validated using samples from the 2-year visit (See Materials and methods), where the same pair of epitopes provided the highest predictive value as in the year 5 OFC (AUC = 73%).

Combining the two principles above resulted in the following algorithm:

If SPT ≤ 3 mm or sIgE ≤ 0.1 kU_A/L then "Not Allergic."
If SPT ≥ 18 mm or sIgE ≥ 18 kU_A/L then "Allergic."
If $\left(\text{Ara h 2\_008} + \frac{\text{Ara h2\_019}}{20}\right) \leq 0.20$ then "Not-Allergic," otherwise "Allergic."

In this specification, low and high values (eg, 3 mm and 18 mm for the SPT) were identified based on previous work.[16] The analytical lower limit of detection was determined (using the Clinical and Laboratory Standards Institute (CLSI) guidelines) for epitopes Ara h 2_008 and Ara h 2_019 and resulted in the decision threshold (0.20). Effectively, if IgE reactivity to either of these two epitopes is sufficiently high, then the result is positive. Moreover, inclusion of additional epitopes did not improve the performance significantly. Considering that a "parsimonious model" is preferable not only for its practicality, but also because it is more likely to avoid overfitting (ie, demonstrate better performance in the development stage than in external validation cohorts or "real life clinical practice"), the algorithm was limited to these two epitopes.

The performance of this algorithm in the LEAP cohort was 91.5%. However, since our algorithm was defined to maximize the performance in LEAP, this was not unexpected. To have a more realistic performance of the diagnostic ability of the peanut BBEA diagnostic test in clinical practice, we needed to evaluate the performance in a validation cohort with a properly documented and locked-down algorithm.

Before validation, and in order to confirm that the peptides were in excess over plasma antibodies, the number of beads per assay was titrated from 100 beads per assay to 3000 beads per assay, and IgE epitope reactivity was determined using peanut-allergic patient samples having high peanut-specific IgE (133.4 kU_A/L) and low peanut-specific IgE (1.26 kU_A/L). In Figure 2, the MFI of the most highly informative epitopes, Ara h2_008 and Ara h2_019 were measured using 100, 300, 1000 or 3000 peptide-bearing beads. In our standard assay, each peptide to be evaluated is present on 1000 beads (master mix). The data demonstrate that there was no significant increase in signal as the number of beads per well was increased from 100 to 3000 beads per well in the low positive control and only a modest 10–15% increase as the number of beads was increased from 300 to 1000 or 3000 beads per well in the high positive control. If the peptide concentrations per well were too low and other immunoglobulin isotypes were "out competing" IgE for specific peptides, we would expect to see a higher IgE mean fluorescence intensity (MFI) in the 3000-bead well compared to the 1000-bead well, which is not the case. This was observed for all peptides in the panel (not shown).

3.2 | Peanut BBEA diagnostic test: Validation

With the laboratory procedures and algorithm for the peanut BBEA diagnostic test locked-down, we tested the performance of the algorithm on the validation cohorts (CoFAR2 and POISED). In order

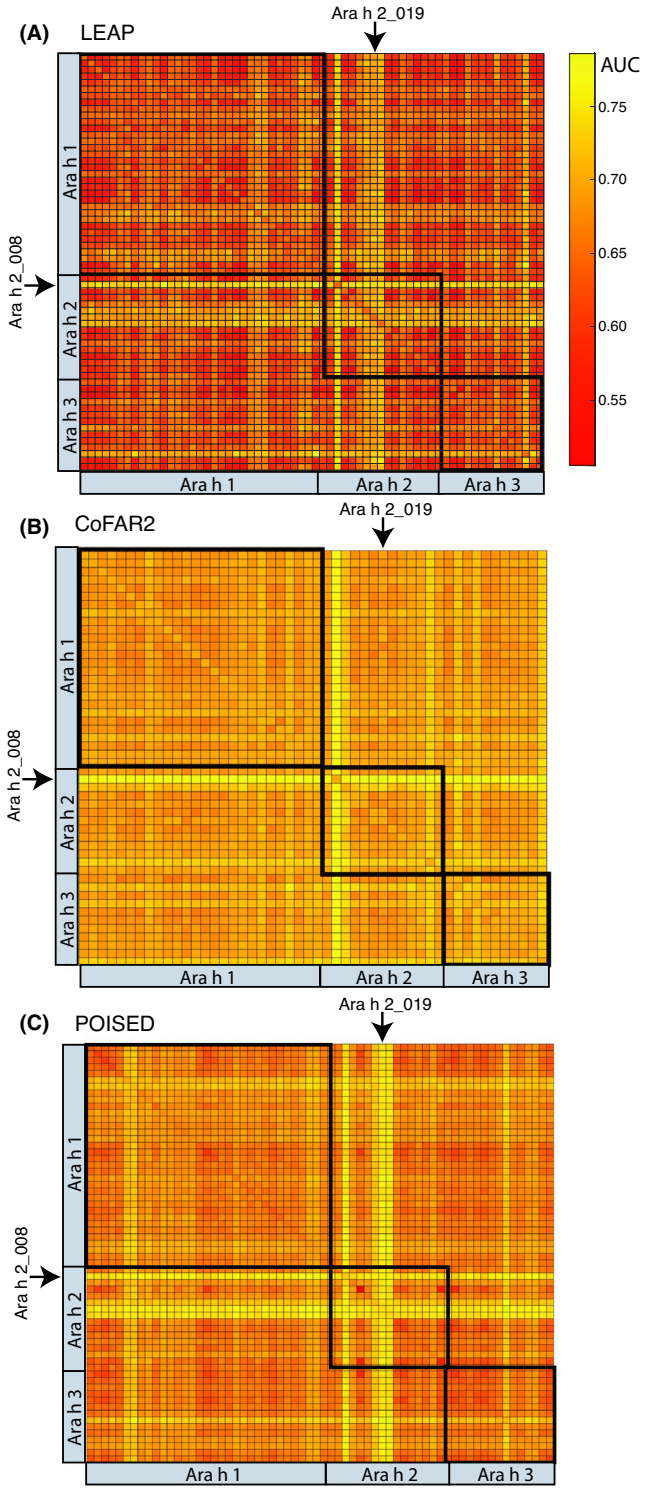

FIGURE 1 AUC heatmaps for all pairs of peanut allergen epitopes for LEAP (A), CoFAR2 (B), and POISED (C). Each heatmap pixel represents the AUC for the logistic regression classifier built over the pair of associated epitopes for classifying allergy status. Colors closer to yellow indicate a higher AUC, with the same color scale for all three plots

to confirm that Ara h 2_008 and Ara h 2_019 were reproducible as the two highest performing epitopes from the same peanut allergen, the AUC was determined for the CoFAR2 and POISED cohorts for all epitope pairs included in the BBEA panel. Figure 1 depicts the pairwise epitope AUC plot for all pairs of epitopes for the LEAP, CoFAR2, and POISED cohorts, demonstrating, which epitope pairs are synergistic, as well as the reproducibility across the discovery and validation cohorts. Pairs of epitopes within the same allergen (Ara h 1, Ara h 2, and Ara h 3) with the highest AUC are within Ara h 2. Specifically, Ara h 2_008 and Ara h 2_019 have the highest combined AUC in both CoFAR2 and POISED, reproducing the discovery observations in LEAP.

To quantify the reproducibility of the peanut BBEA algorithm in the external cohorts, we evaluated its diagnostic performance against the outcome of the DBPCFC for the CoFAR2 and POISED cohorts. Performance measures were derived and presented in Table 2 along with the performance of the other established tests (SPT, peanut sIgE, component proteins). The accuracy of the peanut BBEA was highly significant ($P = .0049$, two-sided McNemar's exact test); the peanut BBEA test had a diagnostic accuracy>91% for both cohorts. In contrast, the commonly used diagnostic thresholds for the serological tests and SPT had accuracies below 74%.

We also evaluated the use of the BBEA diagnostic test without considering sIgE levels to peanut. In this case, the rule in/out step is based on extreme values of SPT followed by the BBEA decision rule. When the performance of the peanut BBEA diagnostic test using only SPT and BBEA markers was evaluated in the two validation cohorts, we observed a decrease in the performance from 93% accuracy to 90% accuracy [95% CI: 84%–94%], but this decrease was not statistically different.

4 | DISCUSSION

With the development of immunotherapeutic approaches to treat peanut allergy coming on line,[20-22] the need for accurate laboratory tests to diagnose peanut allergy as an alternative to oral food challenges in clinical practice will be essential. This will require a diagnostic that has been properly validated, analytically and clinically, with multiple independent cohorts and in the context of a regulated, qualified laboratory to ensure reproducible, reliable results.

We have previously shown that peanut ses-IgE is a promising *prognostic* biomarker of peanut allergy development later in life, using samples from children under 3 years of age to predict their allergy status at 4–11 years of life.[12] In this work, we present a diagnostic test for peanut allergy that could be used at the time of blood draw. The *peanut BBEA diagnostic test* was developed using samples from the LEAP cohort at year 5, where the gold standard OFC was available. The *peanut BBEA diagnostic test* was then validated using 2 independent cohorts POISED and CoFAR2 derived from 2 different countries and different age groups. The *peanut BBEA diagnostic test* has an accuracy of 93% for the diagnosis of peanut allergy, as measured against DBPCFCs in independent cohorts. This exceeds the accuracy of standard available tests, for example, SPT, peanut sIgE and peanut allergen component sIgE tests. Not only is the peanut BBEA substantially more accurate,

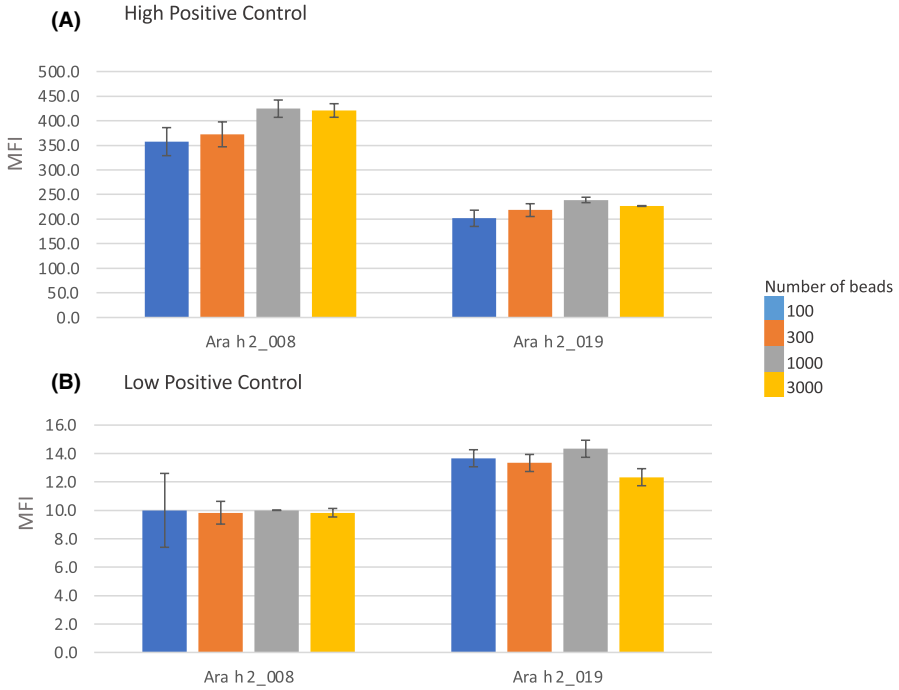

FIGURE 2 Luminex bead titration, 100, 300, 1000, and 3000 beads per well. (A) High positive control (peanut sIgE of 133.4 kU_A/L) IgE reactivity for Ara h2_008 and Ara h2_019 epitopes; (B) Low positive control (peanut sIgE of 1.24 kU_A/L) IgE reactivity for Ara h2_008 and Ara h2_019 epitopes. Mean ± SD across 3 technical replicates

TABLE 2 Performance of Peanut BBEA in Validation on the CoFAR2 and POISED cohorts

Test	Sens.	Spec.	PPV	NPV	FPR	FNR	LR+	LR-	Accuracy (CIs)	AUC
Performance of commonly used thresholds on CoFAR2 cohort										
SPT (n = 82)	95.7%	72.9%	57.9%	97.7%	27.0%	4.0%	3.5	16.8	79.3% (68.9%–87.4%)	84.3%
sIgE (n = 82)	91.3%	42.4%	38.2%	92.6%	58.0%	9.0%	1.6	4.9	56.1% (44.7%–67%)	66.8%
Ara h 1 (n = 80)	34.8%	86%	50%	76.6%	14%	65%	2.5	1.3	71.2% (60–80.8)	60.4%
Ara h 2 (n = 80)	56.5%	80.7%	54.2%	82.1%	19%	43%	2.9	1.9	73.8% (62.7–83)	68.6%
Ara h 3 (n = 80)	13%	87.7%	30%	71.4%	12%	87%	1.1	1	66.2% (54.8%–76.4%)	50.4%
Validation Performance of Peanut BBEA diagnostic test with SPT as rule in/rule out (sIgE excluded)										
COFAR2 (n = 82)	91.3%	88.1%	75.0%	96.3%	12.0%	9.0%	7.7	10.1	89% (80.2%–94.9%)	90%
POISED (n = 84)	83.3%	97.6%	97.2%	85.4%	2%	17%	35	5.9	90.5% (82.1%–95.8%)	90.5%
Combined	86.2%	92.1%	87.5%	91.2%	8%	14%	10.9	6.7	89.8% (84.1%–93.9%)	89.1%
Validation Performance of Peanut BBEA diagnostic test with SPT and sIgE as rule in/rule out										
COFAR2 (n = 82)	91.3%	91.5%	80.8%	96.4%	8.0%	9.0%	10.8	10.5	91.5% (83.2%–96.5%)	91.4%
POISED (n = 84)	92.9%	97.6%	97.5%	93.2%	2%	7%	39	13.7	95.2% (88.3%–98.7%)	95.2%
Combined	92.3%	94.1%	90.9%	95%	6%	8%	15.5	12.2	93.4% (88.5%–96.6%)	93.2%

Note: Performance of SPTs and peanut-, Ara h 1-, Ara h 2-, and Ara h 3-sIgE levels available from the CoFAR2 cohort are presented for comparison. Commonly used thresholds selected to diagnose patients are allergic are SPT >3 mm, sIgE >0.1 kU_A/L, Ara h 1> 0.3 kU_A/L, Ara h 2> 0.3kU_A/L and Ara h 3> 0.3kU_A/L. Thresholds optimizing accuracy with the CoFAR2 cohort were also determined for each serological test and its performance is also displayed. PPV, NPV, FPR, FNR, LR^+, and LR^- are positive predictive value, negative predictive value, false positive rate, false negative rate, positive likelihood ratio and negative likelihood ratio, respectively.

but the false positive rate (FPR) is more than two- to ninefold lower than all other diagnostic tests. It is worth emphasizing that although other studies of diagnostic biomarkers often present results with accuracies greater than 93%, in most cases they refer to the performance of the cohort on which the model was developed (discovery cohort). Such estimates of diagnostic performance typically exceed those that will be observed in actual practice, a phenomenon known as overfitting.[10] The best way to achieve the most realistic estimates of how a diagnostic test will perform in the clinical setting is to validate the algorithm on at least one external cohort in a blinded manner. If this is not feasible, estimates of accuracy obtained using computational techniques like cross-validation or bootstrapping[23,24] should be presented to give the reader a more realistic estimates of the accuracy of a given diagnostic test.

Of note, when the peanut BBEA diagnostic test is used in conjunction with skin testing alone, as commonly done in most allergy practices, there is no significant change in accuracy.

An unexpected discovery presented here is the immunodominance of the Ara h 2 epitopes, Ara h2_008 and Ara h 2_019. Referring to Figure 1 it is apparent that consistently across three cohorts (LEAP, CoFAR2, POISED) Ara h2_008 produces the greatest diagnostic AUC when paired with any other epitope from Ara h 2, and in particular Ara h2_019. While it is not surprising that Ara h 2 epitopes are dominant based on previous studies,[3,5,25,26] our data suggest that 95% of all reactions involve crosslinking of Ara h 2_008 or Ara h 2_019 with any other epitope in Ara h 2.

One limitation of this study includes the fact that the cohorts used for discovery and validation were not specifically designed to investigate the predictive value of the diagnostic test and to prospectively study the diagnostic accuracy of peanut BBEA. However, since food allergy is most prevalent in children, such a study in children would not be ethically and logistically feasible. Instead, we have taken advantage of available cohorts in which the diagnosis of peanut allergy was confirmed by oral food challenge and validated our algorithm with two independent patient populations.

In summary, when compared to the "gold standard" DBPCFC, the peanut BBEA diagnostic test provides the most accurate *in vitro* diagnostic test for peanut allergy developed to date. This high throughput assay requires less than 0.1ml of plasma/serum and is easily adaptable to the standard clinical lab. With the recent approval of Palforzia® in the USA and Europe for the treatment of peanut allergy and the presence of several other therapeutics in the pipeline, the availability of a highly accurate blood test to establish the diagnosis of peanut allergy will greatly facilitate patient selection for therapeutic intervention. Recent studies evaluating the peanut epitopes also suggest that this assay may enable clinicians to predict a patient's eliciting dose of peanut and the potential severity of a reaction in case of accidental ingestion, but further investigation is needed to validate these studies.[27,28]

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

MSF, MS, PK, BG, and HS: involved in the design, analysis, and manuscript preparation. DL, MW, GG: performed the epitope assays. CH and AP: done the analysis of bead titrations. GdT, GL, SC, SG, and KN: provided patient samples. All authors reviewed and approved the manuscript.

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