

Allergen components in diagnosing childhood hazelnut allergy: Systematic literature review and meta-analysis

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Abstract

Background: Hazelnut-specific IgE antibodies (slgEs) in serum support the diagnosis of hazelnut allergy, but extract-based tests have low diagnostic specificity, commonly leading to over-diagnosis. Measuring sensitization to individual allergen components may enhance the diagnosis of hazelnut allergy. We systematically examined data on diagnostic accuracy of slgE to commercially available hazelnut components to compare their individual contributions in diagnosing hazelnut allergy.

Methods: Seven databases were searched for diagnostic studies on patients suspected of having hazelnut allergy. Studies employing component-specific IgE testing on patients whose final diagnosis was determined by oral food challenges were included in the meta-analysis. Study quality was assessed as recommended by Cochrane.

Results: Seven cross-sectional studies and one case-control study were identified, seven presenting data on children (N = 635), and one on a mixed age population. Overall, the diagnostic accuracies of slgE to both Cor a 9 and Cor a 14 were significantly higher than for Cor a 1-slgE ($P < .05$). In children, the specificity of Cor a 14-slgE at 0.35 kU_A/L cutoff was 81.7% (95% CI 77.1, 85.6), and 67.3% (60.3, 73.6) for Cor a 9-slgE. The specificities for Cor a 1-slgE and hazelnut-slgE were 22.5% (7.4, 51.2) and 10.8% (3.4, 29.8), respectively. The sensitivity of Cor a 1-slgE (60.2% [46.9, 72.2]) was lower than for hazelnut extract-slgE (95.7% [88.7, 98.5]), while their specificities did not differ significantly.

Conclusion: slgE to Cor a 14 and Cor a 9 hazelnut storage proteins increases diagnostic specificity in assessing hazelnut allergy in children. The combined use of hazelnut extract and hazelnut storage proteins may improve diagnostic value.

KEYWORDS

allergen, children, component-resolved diagnostics, Cor a 1, Cor a 14, Cor a 9, hazelnut, storage protein

1 | INTRODUCTION

Tree nut allergies affect 1.2% of children and adults,^{1,2} and hazelnut allergy is among the most common of these allergies. Symptoms

range from mild oral symptoms to severe systemic reactions.^{3,4} Children with hazelnut allergy often react with generalized symptoms, while adults more frequently experience localized oral symptoms.^{5,6}

Hazelnut allergy diagnosis is based on a clinical history supported by positive results from IgE sensitization tests.⁷ Extract-based hazelnut tests are sensitive, but moderate predictors of allergy.^{8,9} Therefore, alternative methods that could prevent over-diagnosis and reduce the number of oral food challenges (OFC) are attractive. The current gold standard for diagnosing food allergy is the double-blind placebo-controlled food challenge (DBPCFC), a time-consuming and costly procedure. In clinical practice, few food challenges are performed and the majority are not blinded, to save time and resources.³

Component-resolved diagnostics (CRD) measure sIgE to individual proteins and can distinguish between sensitization to specific and cross-reactive components.¹⁰ The value of CRD has been demonstrated in peanut allergy, where sensitization to storage proteins is associated with systemic allergic reactions, and sensitization to cross-reactive PR-10 proteins predominantly causes mild, oral reactions.¹¹ In hazelnut allergy, evidence for a similar contribution of CRD to clinical diagnosis has been shown. Readily available hazelnut component tests include storage proteins Cor a 9 and Cor a 14, and the cross-reactive proteins Cor a 1 (PR-10) and Cor a 8 (lipid transfer protein, LTP).^{12,13} Other hazelnut components are the 7S-vicilin-like protein Cor a 11¹⁴ and two oleosins (Cor a 12 and Cor a 13), to which sensitization has been demonstrated, although their clinical relevance has not been confirmed.¹²

With the objective to assess the quality and consistency of evidence that available components are helpful in reducing over-diagnosis and the need for oral food challenges for a final diagnosis of hazelnut allergy, we undertook a systematic literature review to compare, whenever possible or meaningful, the diagnostic accuracy of hazelnut storage protein components Cor a 9 and Cor a 14 with those of the cross-reactive components Cor a 1 and Cor a 8 in patients suspected of hazelnut allergy based on previous reactions or sensitization to hazelnut extracts.

2 | METHODS

2.1 | Search strategy

We systematically searched seven electronic databases (Medline, Medline In-Process, Embase, PubMed, Cochrane Central Register of Controlled Trials, NIH Clinicaltrials.gov, WHO International Clinical Trials Registry Platform) for records published prior to October 3, 2018, using the search terms "peanut hypersensitivity" or "nut hypersensitivity" as index terms with peanut* or groundnut* or hazelnut* or cobnut* or filbert* and allerg* or sensitivit* or hypersensitivit* or reaction in the title or abstract, and with "immunoglobulin e" as an index term or "immunoglobulin e" or "sIgE" in the abstract or title. A first literature search was carried out up to January 22, 2016, and an updated literature search was done to October 3, 2018. Complementary hand searching of reference lists of selected systematic reviews published between 2011 and October 3, 2018 and relevant allergy journal websites was also undertaken. The detailed search strategy is available on request.

Key Message

Extract-based sIgE tests are poor predictors of hazelnut allergy: over-diagnosis is common. Component-based diagnostics may improve specificity, distinguishing clinically relevant sensitizations from those of lower relevance. Our meta-analysis shows that sIgE to Cor a 9 and Cor a 14 are of high diagnostic accuracy for hazelnut allergy in children, whereas the cross-reactive Cor a 1 has low diagnostic accuracy. This study may impact on the diagnosis of hazelnut allergy in children: testing for sIgE to hazelnut components can help identify patients with clinically relevant hazelnut allergy, reduce over-diagnosis, and may lead to reduced need of oral food challenges in children.

2.2 | Inclusion, exclusion criteria, and data extraction

Studies meeting pre-defined eligibility criteria published in English between January 1, 2010 and October 3, 2018, including at least 15 patients of any age that were suspected of hazelnut allergy based on previous reaction to hazelnut and/or sensitization to hazelnut extracts, were included. Studies were required to contain sIgE measurements to at least one hazelnut allergen component (Cor a 1, Cor a 8, Cor a 9, Cor a 14), presenting data for calculation of sensitivity and specificity. The reference standard was OFC (blind or open); exceptions included patients classified as hazelnut allergic based on recent anaphylaxis or other convincing evidence confirmed by an allergist. A minimum of 50% of patients must have received an OFC. Conference abstracts, case reports, case series, editorials, and reviews were excluded. Two independent external analysts reviewed full-text articles and disagreements were resolved through discussion.

Extracted data were summarized into 2 × 2 tables describing index test results at each reported threshold. The final dataset (Table S1) covers data extracted during the systematic literature review, data on file on all hazelnut components received from Dr Brandström and Dr Eller,^{15,16} and Cor a 1 and Cor a 9 data manually extracted from the study by Masthoff et al¹⁷ using graphical software.¹⁸ Studies reported test result data using different thresholds for positivity; a meta-analysis that includes studies with mixed thresholds will produce an estimate relating to an unspecified average threshold that is not clinically meaningful.¹⁹ Therefore, our meta-analysis included hazelnut component data reported at defined cutoffs: 0.1, 0.35, 1.0, or 5.0 kU_A/L. Hazelnut extract data at 0.35 kU_A/L were used as a reference for results interpretation.

The reviewers assessed risk of bias of the included studies using 11 quality items listed in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy,²⁰ plus two additional quality items of importance for this review ("Were cutoff values established before the study was started?" and "Was the study free of commercial funding?"). As per Cochrane recommendations, a benchmarking

exercise was conducted prior to the quality assessment to determine the minimum criteria for each quality item. Quality items were coded yes if they met the benchmark criteria, no if they did not, or unclear if there was insufficient information to make a judgment.²⁰

2.3 | Diagnostic accuracy determination—statistical analyses

The primary analysis included all studies meeting the inclusion criteria at the pre-defined 0.35 kU_A/L threshold. From the constructed 2 × 2 tables, estimates of study-level sIgE sensitivity and specificity were calculated with 95% confidence intervals (CIs), and the study-level data were summarized as paired forest plots. Subanalyses focused on pediatric patients and were performed at the thresholds applied in the retrieved studies: 0.10, 0.35, 1.0, and 5.0 kU_A/L.

For each analysis and threshold, a random-effect meta-analysis model using the Reitsma bivariate method²¹ was run to estimate the tests' pooled sensitivities, pooled specificities, and area under the curve (AUC). Outcomes for the study-level pooled estimates were presented as forest plots. The pediatric subanalyses were presented as forest plots and as summary receiver-operating characteristic (sROC) plots. From sROC analyses, the tests' diagnostic accuracies were considered significantly different if there was no overlap of the 95% CIs ellipses. Diagnostic odds ratios (DOR) and positive and negative likelihood ratios (LR+/LR-) were calculated; LRs 95% CIs were calculated using the Log method.²² In every analysis, heterogeneity among studies was assessed with the Higgins *I*² test,²³ which is the total between-study variance divided by the total variance expressed as a percentage; when *I*² is lower than 40%, the heterogeneity between studies is considered low.²⁴

The analyses were performed with the "Mada" package of the statistical software R (version 3.3.0)^{25,26} to fit all analyses.²¹ The statistical significance level was set at .05.

2.4 | Estimation of predicted clinical implications

The clinical implications of the key findings from the diagnostic meta-analyses were calculated and projected using the average disease prevalence from the included pediatric studies to estimate the percentage of children who would be accurately diagnosed with hazelnut components at 0.10, 0.35, and 1.0 kU_A/L cutoffs. Results were grouped as "correctly classified" (True Positives [TPs] + True Negatives [TNs]) – or "incorrectly classified" (False positives [FPs] + False Negatives [FNs]).

3 | RESULTS

3.1 | Study characteristics and methodological quality

The systematic literature search identified 2225 unique citations; 2025 citations were excluded based on the title and abstract,

leaving 200 full-text publications for a second review. Finally, eight studies on the accuracy of hazelnut component sIgE antibody for the diagnosis of hazelnut allergy were relevant for inclusion in the meta-analysis (Figure 1A).^{15-17,27-31} Of these, seven were cross-sectional and one had a case-control design (Table 1), and presented data from Europe (*n* = 7) and the United States (*n* = 1). Six of the included studies recruited pediatric patients only, two included both children and adults,^{17,29} one of which presented these data separately.¹⁷ At the 0.35 kU_A/L threshold, seven studies reported data for Cor a 14-sIgE,^{15-17,27-30} five Cor a 9 sIgE,^{15-17,29,30} while four studies reported the diagnostic accuracy of Cor a 1-sIgE (Table 1).^{15-17,29} Data on sIgE to Cor a 8 at 0.35 kU_A/L were only reported in two studies (both enrolling children and adults, Table 1).^{17,29} In all studies, sIgE antibody levels were determined using the same assay platform (ImmunoCAP™).

No concerns regarding the applicability of the studies were found (Figure 1B). All studies were marked down for the quality item "Differential verification avoided" apart from Brandström et al.¹⁵ This was because not all patients received the same reference standard: Some patients were diagnosed based on the results of an OFC, whereas others were diagnosed based on clinical history or previous anaphylaxis. This does not imply that the methodological quality of these studies is low, as it is standard practice not to challenge all patients. The complete assessment is detailed in (Table S2).

3.2 | Diagnostic accuracy determination—meta-analyses

3.2.1 | Primary analysis: all included studies

Data from studies using 0.35 kU_A/L as the cutoff threshold for Cor a 1 (*n* = 4 studies), Cor a 9 (*n* = 5), and Cor a 14 (*n* = 7) were included in the primary analysis. Figure 2A shows forest plots for sensitivity and specificity summarizing the overall diagnostic characteristics of the four component tests in diagnosing hazelnut allergy per included study. The sensitivity and specificity results were consistent across studies except for one study that recruited both children and adults,²⁹ where the sensitivity of Cor a 9 and Cor a 14 was significantly lower than in other studies (based on non-overlapping 95% CIs). Similarly, for the adult subset in the Masthoff et al study,¹⁷ sensitivities of Cor a 9-sIgE and Cor a 14-sIgE were lower than the pediatric studies. The pooled sensitivities of the three hazelnut component sIgEs were 64.6% (Cor a 9), 65.1% (Cor a 14), and 72.7% (Cor a 1) and did not differ significantly from each other (Figure 2A). In contrast, the pooled specificities of Cor a 9 (74.1% [63.2, 82.6]) and Cor a 14-sIgE (84.9% [79.4, 89.2]) were significantly (*P* < .05) higher than for Cor a 1-sIgE 20.3% ([7.9, 43.0]).

Compared to the pooled sensitivity of hazelnut extract-sIgE (95.4% [89.4, 98.0], data not shown), all three component sIgEs were significantly less sensitive (*P* < .05) (Figure 2A), while the pooled specificities of both Cor a 9 and Cor a 14 were significantly higher (*P* < .05) (data not shown).

FIGURE 1 Study characteristics. A, PRISMA flow diagram of included studies. B, Risk of bias and applicability concerns. Reviewer judgments on 13 quality items presented as percentages across included studies [Colour figure can be viewed at wileyonlinelibrary.com]

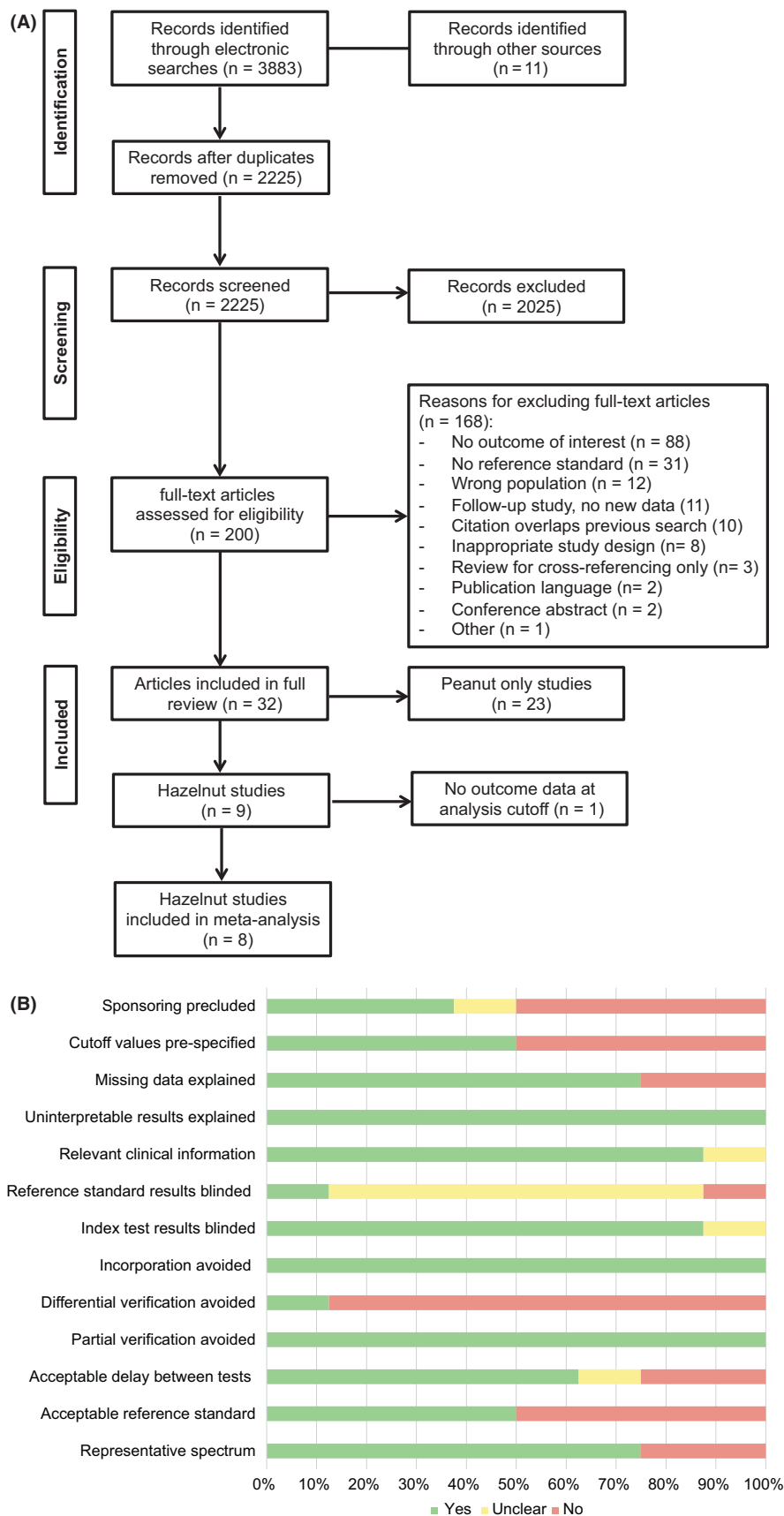


TABLE 1 Summary of included study characteristics

Study	Country	Reference standard	Age of included subjects, years (median; span)	N patients (allergic/ tolerant)	Hazelnut sIgE test components/ extracts	Reported cutoffs of relevance (kU _A /L)
Beyer et al (2015) ²⁷	Germany	DBPCFC or open OFC	4.3 for reacting 4.7 for tolerant (2.1-8.1)	143 (44/99)	Cor a 14 Hazelnut extract	0.1, 0.35, 1.0 0.35
Brandström et al (2015) ¹⁵	Sweden	DBPCFC	11 (6-18)	40 (8/32)	Cor a 1 Cor a 9 Cor a 14 Hazelnut extract	0.1, 0.35, 1.0 0.1, 0.35, 1.0 0.1, 0.35, 1.0 0.35
Buyuktiryaki et al (2016) ²⁸	Turkey	DBPCFC or recent history of anaphylaxis	3.4 (2.1-7.2)	64 (32/32)	Cor a 1 Cor a 9 Cor a 14 Cor a 8 Hazelnut extract	0.1 0.1 0.35, 1.0, 5.0 0.1 0.35
Datema et al (2015) ²⁹	Spain, Bulgaria, Iceland, Greece, Czech Republic, Poland, The Netherlands, France, United Kingdom, Italy, Switzerland, and Lithuania	DBPCFC or recent history of anaphylaxis	NR; (3-55) ^a	129 (109/20)	Cor a 1 Cor a 9 Cor a 14 Cor a 8	0.35 ^b 0.35 ^b 0.35 ^a 0.35 ^b
Carraro et al (2016) ³¹	Italy	DBPCFC or history of objective symptoms with ingestion of hazelnut	NR; (3-14)	36 (24/12)	Cor a 9	1.01
Eller et al (2016) ¹⁶	Denmark	DBPCFC or open OFC	5.1 (0.7-15.5) ^a	155 (65/90)	Cor a 1 ^c Cor a 9 ^c Cor a 14	0.1, 0.35, 1.0, 5.0 0.1, 0.35, 1.0, 5.0 0.1 ^c , 0.35, 1.0 ^c , 5.0 ^c
Kattan et al (2014) ³⁰	USA	Open OFC or history of objective symptoms with ingestion of hazelnut	9.0 (3.5-13.0)	116 (9/107)	Cor a 1 Cor a 9 Cor a 14 Cor a 8 Hazelnut extract	0.1 0.35, 1.0, 5.0 0.35, 1.0, 5.0 0.1 0.1
Masthoff et al (2013) ¹⁷	The Netherlands	DBPCFC	children: 8 (5-12) adults: 29 (22-45)	81 (40/41) 80 (39/41)	Cor a 1 Cor a 9 Cor a 14 Cor a 8 Hazelnut extract	0.35, 1.0 0.35, 1.0, 5.0 0.35, 1.0, 5.0 0.35 0.35

Abbreviations: DBPCFC, double-blind placebo-controlled food challenge; NR, not reported; OFC, oral food challenge.

^aUnclear whether mean or median.

^bMixed adult/child population (excluded from subanalyses).

^cData on file.

3.2.2 | Subanalyses on cross-sectional pediatric studies

A subanalysis was undertaken on pediatric studies because six of the seven studies reported diagnostic accuracy data in children at the commonly used clinical threshold of 0.35 kU_A/L (Table 1).^{15-17,27,28,30} Pooled

sensitivities and specificities for the three different hazelnut component sIgEs are shown in Figure 2B. Based on pediatric studies, pooled sensitivities of sIgE to the components Cor a 1, Cor a 9, and Cor a 14 ranged from 60.2% to 77.9%. The sensitivity of Cor a 1-sIgE was lower compared to Cor a 9 and Cor a 14, but this did not reach statistical significance. With respect to specificity, however, sIgE to Cor a 14 was

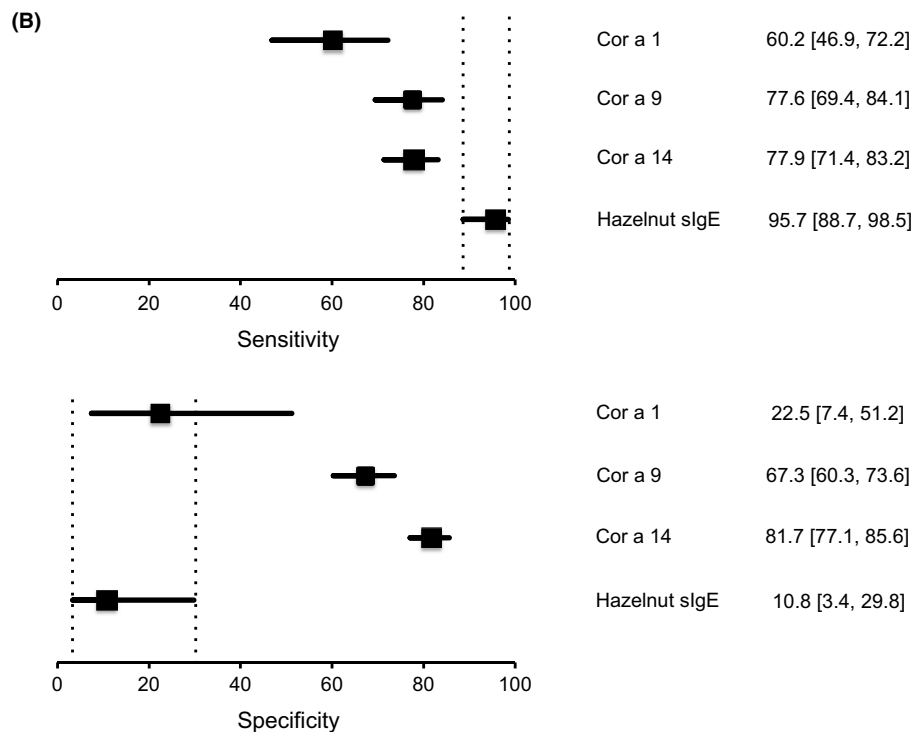
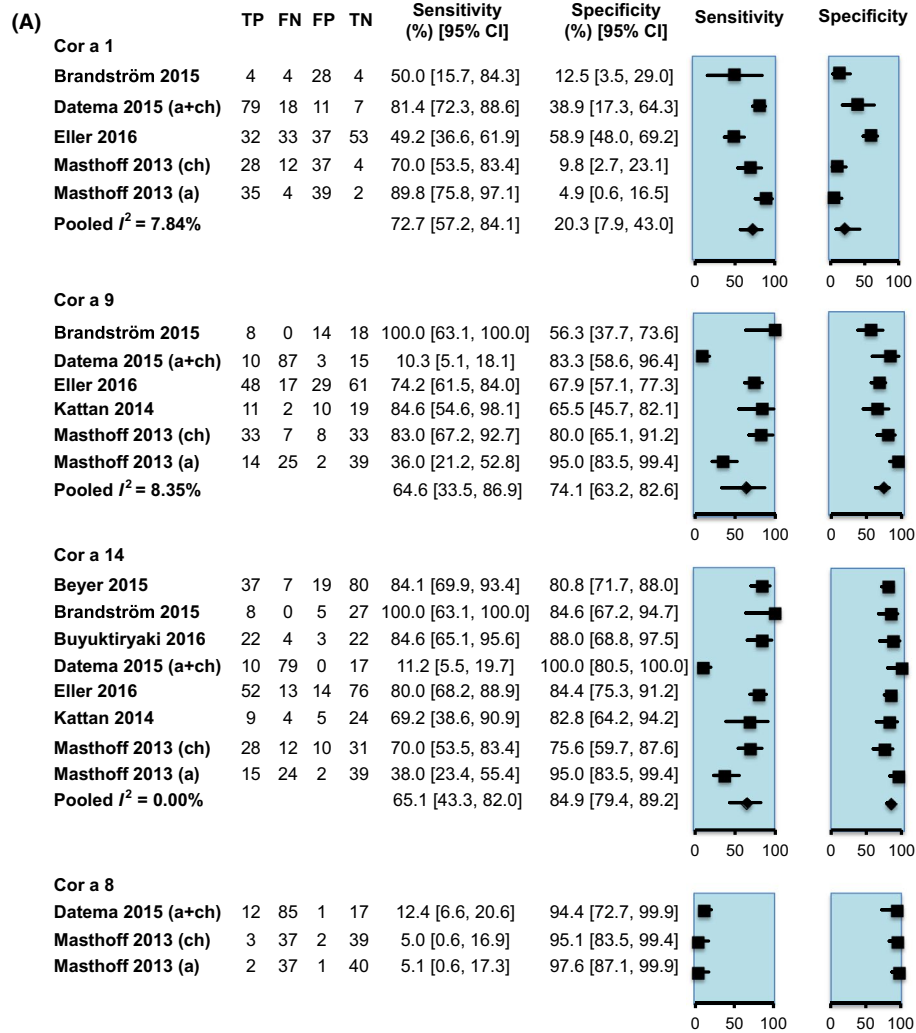


FIGURE 2 Diagnostic accuracy of sIgE to hazelnut components. A, Sensitivity and specificity of sIgE to hazelnut components per included study and pooled values, where applicable (primary analysis: 0.35 kU_A/L cutoff) (a), adults; (ch), children; FN, false negatives; FP, false positives; TN true negatives; TP, true positives. B, Pooled estimates of sensitivity and specificity for hazelnut component sIgE and sIgE to hazelnut extract at 0.35 kUA/L threshold in children (subanalysis) Based on data from^{15-17,27,28,30} [Colour figure can be viewed at wileyonlinelibrary.com]

significantly higher (81.7% [77.1, 85.6]) than Cor a 9 (67.3% [60.3, 73.6], $P < .05$), and sIgE to both Cor a 9 and Cor a 14 was significantly better at ruling out hazelnut allergy than Cor a 1 (22.5% [7.4, 51.2], $P < .05$) (Figure 2B). Hazelnut extract-sIgE showed significantly higher sensitivity (95.7% [88.7, 98.5]) than all component sIgEs ($P < .05$), while its specificity (10.8%) did not differ significantly from that of Cor a 1-sIgE. Overall, the heterogeneity of data in the pediatric studies, as judged by the I^2 value, was low. Only for sIgE to Cor a 1, I^2 was moderately increased (30%-40% in the various analyses, still considered acceptable²⁴).

Where sufficient data for meta-analyses were available, the diagnostic accuracy (sensitivity, specificity, AUC, DOR, and LR+/LR-) was calculated for hazelnut component sIgE at four cutoff levels (Table 2). Figure 3 shows the sROC curves of the pooled sensitivities and specificities of Cor a 1, Cor a 9, and Cor a 14-sIgE at the 0.1, 0.35, and 1.0 kU_A/L cutoffs. Results at the 5.0 kU_A/L threshold are depicted in the Figure S1.

The specificities of sIgE to Cor a 9 and Cor a 14 were significantly lower at 0.10 kU_A/L than at 0.35 kU_A/L ($P < .05$), while no difference in sensitivity was observed between the 0.10 and 1.0 kU_A/L cutoffs (Figure 3, Table 2). As expected, the sROC curves showed an inverse

correlation between sensitivity and specificity (a decrease in sensitivity correlated to an increase in specificity), though this correlation was weak for Cor a 1 (Figure S1). For Cor a 14-sIgE, an increase in the threshold from 0.1 to 5.0 kU_A/L resulted in a decrease in sensitivity from 85.5% to 53.2%, while the specificity increased from 61.5% to 97.3% (Table 2). Correspondingly, the sensitivity of Cor a 9-sIgE decreased from 86.0% to 43.1%, while the specificity increased from 42.6% to 89.6%.

The specificity of IgE to Cor a 14 at both 0.10 and 0.35 kU_A/L (61.5% and 81.7%, respectively) was significantly higher than that of Cor a 9-sIgE at the same cutoffs (42.6% and 67.3%, respectively $P < .05$), while the sensitivities were not significantly different between these two tests at either of the cutoffs. At the 1.0 kU_A/L threshold, no differences in sensitivity or specificity between sIgE to Cor a 9 and Cor a 14 were found (Table 2), as indicated by the overlapping 95% CI ellipses (Figure 3).

The combined sensitivity and specificity as reflected by the AUC showed that Cor a 14-sIgE had the highest diagnostic accuracy at 0.35 kU_A/L cutoff, while the highest diagnostic accuracy for Cor a 9-sIgE was at 1.0 kU_A/L. The positive and negative likelihood ratios for Cor a 14-sIgE at 0.35 kU_A/L were 4.26 and 0.27, respectively, and

TABLE 2 Summary of meta-analysis results for each hazelnut component sIgE in children

Test/Threshold (kU _A /L)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC	DOR (95% CI)	LR+ (95% CI)	LR- (95% CI)
Cor a 1						
0.10	48.5 (25.4, 72.3)	49.5 (11.7, 87.8)	0.48	0.84 (0.24, 2.91)	0.96 (0.76, 1.22)	1.04 (0.83, 1.31)
0.35	60.2 (46.8, 72.2)	22.5 (7.4, 51.2)	0.50	0.41 (0.11, 1.58)	0.78 (0.66, 0.92)	1.77 (1.23, 2.55)
1.0	54.9 (43.9, 65.4)	28.0 (8.1, 63.2)	0.51	0.46 (0.08, 2.47)	0.76 (0.63, 0.93)	1.61 (1.17, 2.22)
Cor a 9						
0.10	86.0 (74.1, 93.0)	42.6 (34.5, 51.2)	0.57	4.41 (1.32, 14.70)	1.50 (1.29, 1.74)	0.33 (0.20, 0.54)
0.35	77.6 (69.4, 84.1)	67.3 (60.3, 73.6)	0.77	8.62 (4.77, 15.58)	2.37 (1.90, 2.97)	0.33 (0.24, 0.47)
1.0	71.5 (63.3, 78.5)	78.7 (72.1, 84.1)	0.82	14.19 (5.67, 35.53)	3.36 (2.53, 4.45)	0.36 (0.28, 0.47)
5.0	43.1 (34.8, 51.8)	89.6 (83.9, 93.5)	0.65	8.72 (2.98, 25.49)	4.14 (2.61, 6.57)	0.64 (0.54, 0.75)
Cor a 14						
0.10	85.5 (78.8, 90.3)	61.5 (55.6, 67.0)	0.76	9.63 (5.51, 16.86)	2.22 (1.89, 2.61)	0.24 (0.16, 0.35)
0.35	77.9 (71.3, 83.2)	81.7 (77.1, 85.6)	0.83	16.45 (9.54, 28.36)	4.26 (3.33, 5.44)	0.27 (0.21, 0.35)
1.0	71.9 (65.2, 77.8)	87.3 (80.3, 92.1)	0.76	20.36 (9.25, 44.80)	5.66 (4.19, 7.67)	0.32 (0.26, 0.40)
5.0	53.2 (45.3, 60.9)	97.3 (93.9, 98.8)	0.62	42.76 (16.75, 109.13)	19.70 (8.74, 44.40)	0.48 (0.41, 0.57)
Hazelnut extract sIgE						
0.35	95.5 (87.5, 98.4)	11.1 (3.4, 30.7)	0.81	3.13 (1.17, 8.38)	1.07 (1.00, 1.15)	0.41 (0.16, 1.04)

Note: Cor a 1: 0.10 kU_A/L data from^{15,16,28,30}; 0.35 kU_A/L data from^{15-17,29}; 1.0 kU_A/L data from¹⁵⁻¹⁷; Cor a 9: 0.10 kU_A/L data from^{15,16,28,30}; 0.35 kU_A/L data from^{15-17,30}; 1.0 kU_A/L data from^{15-17,30,31}; Cor a 14: 0.10 kU_A/L data from^{15,16,27,28,30}; 0.35 kU_A/L data from^{15-17,27-30}; 1.0 kU_A/L data from^{15-17,27,28,30}

Abbreviations: AUC, area under the curve; CI, confidence interval; DOR, diagnostic odds ratio; LR-, negative likelihood ratio; LR+, positive likelihood ratio.

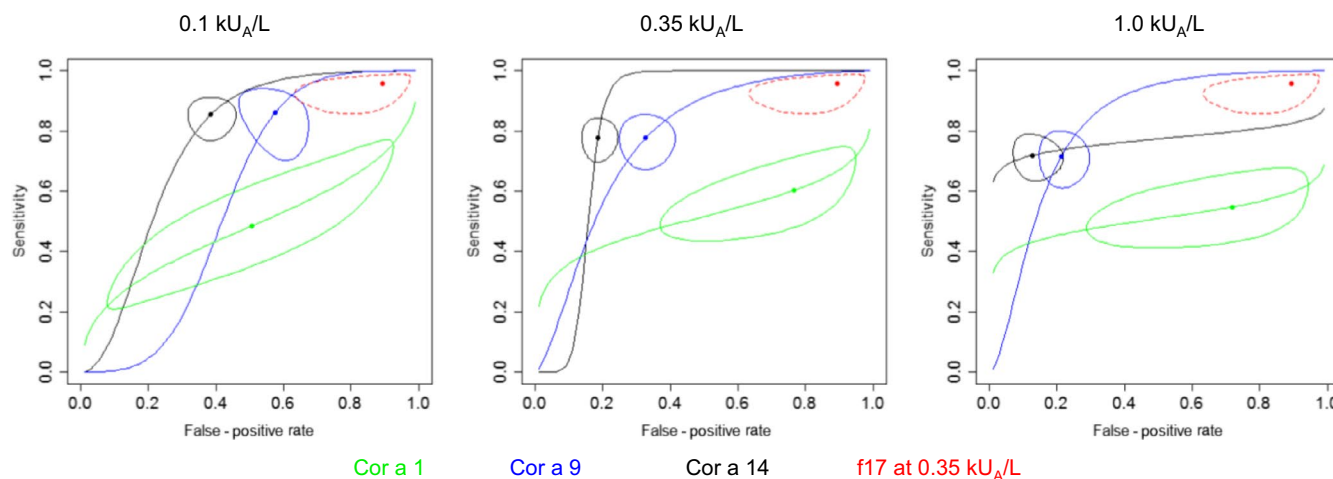


FIGURE 3 SROC curves comparing sIgE to hazelnut components at thresholds of 0.10, 0.35, and 1.0 kU_A/L in children. Ellipses identify the 95% CI region. Pooled sensitivities and specificities calculated with the Reitsma bivariate model on the sROC plot. Diagnostic performance data for hazelnut extract (f17) sIgE at the 0.35 kU_A/L cutoff are shown as comparator (red symbol with dotted line). See Table 1 for included studies [Colour figure can be viewed at wileyonlinelibrary.com]

for Cor a 9-sIgE at 1.0 kU_A/L 3.42 and 0.37, respectively (Table 2). The DOR for Cor a 14 at 0.35 kU_A/L was 20.36 while that of Cor a 9 at 1.0 kU_A/L was 16.96 (Table 2).

For Cor a 1-sIgE, the diagnostic performance was not significantly different across thresholds, and the AUC ranged from 0.48 to 0.56, indicating low diagnostic accuracy. This result was also reflected both by the shape of the sROC curves and the off-scale LR+ (0.78) and LR- (1.77) values (Table 2, Figure 3), which reveal that the test is uninformative¹⁹ for the diagnosis of hazelnut allergy.

3.3 | Clinical implications of key findings

Based on the diagnostic performance and prevalence of hazelnut allergy in the retrieved pediatric studies included in the meta-analysis (35.0% [222/635] pediatric patients were allergic), the diagnostic accuracy of the three available hazelnut component sIgE tests was projected (Figure 4). The lowest number of FNs was found at 0.1 kU_A/L for both Cor a 9-sIgE (4.9%) and Cor a 14-sIgE (5.1%), where the number of FPs was 37.3% and 25.0%, respectively. At 1.0 kU_A/L , 76.2% and 81.9% of patients would be correctly diagnosed (TP + TN) by Cor a 9-sIgE and Cor a 14-sIgE, respectively. At the threshold of 0.35 kU_A/L , slightly fewer children would be correctly diagnosed (70.9% and 80.4% and for Cor a 9 and Cor a 14, respectively), but with a reduced number of FPs for Cor a 14 (Figure 4). As expected, the diagnostic capacity of Cor a 1-sIgE was considerably lower, with 35.7% of children correctly diagnosed at 0.35 kU_A/L (50.4% FPs and 13.9% FNs).

4 | DISCUSSION

Our aim was to compare the diagnostic accuracy of sIgE to available hazelnut storage proteins and the pollen cross-reactive PR-10 Cor a

1 and LTP Cor a 8 proteins in patients suspected of hazelnut allergy through a systematic approach. As seven of eight eligible studies reported pediatric data, the tailored meta-analyses were performed by threshold on pediatric studies. The results show that sIgE to hazelnut storage proteins, Cor a 14 and Cor a 9, confers higher specificity in the diagnosis of hazelnut allergy in children compared with Cor a 1 or hazelnut extract, and could be used clinically to improve the identification of allergic children and reduce the number of confirmatory food challenges. We decided not to quantify the contribution of sIgE to Cor a 8 due to limited data.

Cor a 14-sIgE was found to be the best discriminator of hazelnut allergy, as judged by OFC outcome. Similarly, Cor a 9-sIgE had high specificity, but a somewhat lower overall diagnostic accuracy. The sensitivity and specificity of both Cor a 9 and Cor a 14-sIgE were consistent across pediatric studies, and the diagnostic parameters calculated showed low data variation. This likely reflects the role of storage proteins as early sensitizers and markers of hazelnut allergy.³²⁻³⁴ The results presented are the first meta-analysis data focusing on hazelnut allergen components, but a previous systematic review on CRD in food allergies³⁵ and studies on peanut and other tree nut allergies (cashew and walnut) together show that the respective 2S albumins have high accuracy in identifying children with allergies and objective symptoms.³⁶⁻³⁹

In comparison, the diagnostic accuracy of sIgE to Cor a 1 was lower, as seen by the shape and slope of the sROC curves, and the AUC close to 50% at all three thresholds studied. This reflects the cross-reactive nature of Cor a 1-sIgE, where sensitization is driven by birch or birch-related tree pollen allergies^{3,40} rather than primary hazelnut allergy. The diverse geographic locations of the studies as well as age differences may contribute to the data variation, as pollen cross-reactive sensitizations become increasingly common when children reach school age.^{5,33,34,41} The evaluation of sIgE to Cor a 8, the hazelnut LTP, was hampered by the scarcity of data (only two studies on mixed adult and child populations reported data).

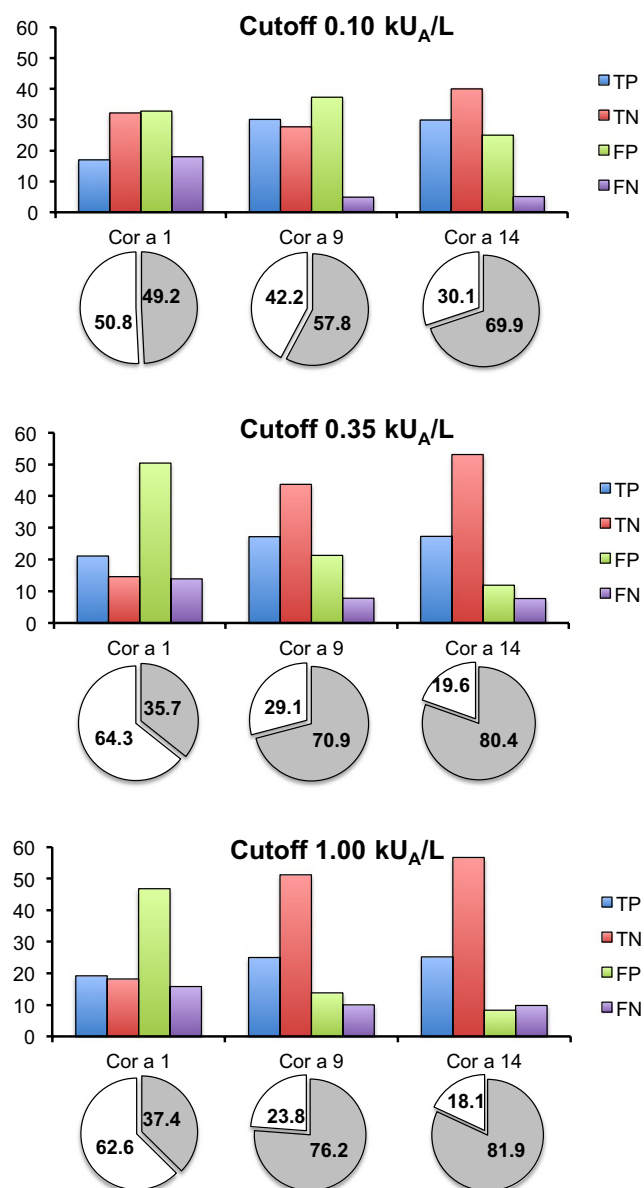


FIGURE 4 Diagnostic outcome of sIgE to hazelnut components in children at thresholds 0.10, 0.35, and 1.0 kU_A/L using the disease prevalence of included studies (222/635, 35.0%). Histograms show percent false negative (FN), false positive (FP), true negative (TN), and true positive (TP), and pie charts depict percentage of correctly diagnosed patients (gray) vs misdiagnosed patients (white). Y-axes represent percentages; histograms show TPs (blue), TNs (red), FPs (green), and FNs (purple). In the pie charts, the gray slice represents the percentage of correctly diagnosed individuals (TPs + TNs), while the white slice represents the percentage of incorrectly classified individuals (FPs + FNs) [Colour figure can be viewed at wileyonlinelibrary.com]

Hazelnut extract-based test results from included articles were used as a comparator for results interpretation. High sensitivity of these tests was confirmed; the pooled sensitivity calculated may, however, be over-estimated, as hazelnut sensitization was an inclusion criterion in some studies. Since hazelnut allergy can be potentially severe, it is important to avoid under-diagnosis, while

over-diagnosis may impair patient quality of life.⁴² We addressed diagnostic cutoff levels for optimal balance of over- and under-diagnosis. In a population with the same disease prevalence as in the included studies (35.0%), Cor a 14-sIgE at the threshold of 0.35 kU_A/L would correctly diagnose 80.4% of children, striking the best balance between false-negative and false-positive results. However, as very low levels of sIgE to primary allergens can also be relevant,⁴³ especially in young children,⁴⁴ it may be important to quantify sIgE levels to the limit of detection (0.1 kU_A/L),⁴⁵ and to carefully review the clinical history of each patient. Therefore, these tests (Cor a 9 and Cor a 14) should be used in association with limit the risk of misclassifying patients.

The diagnostic accuracy of step-wise sIgE to Cor a 14 and Cor a 9 tests could not be evaluated, as they cannot be considered independent tests. However, children sensitized to Cor a 9 in the absence of sensitization to Cor a 14 are reported to react with objective symptoms.^{16,32} Thus, ruling out sensitization to both these storage proteins in children suspected of hazelnut allergy is important. Further, combined testing for sIgE to Cor a 9 and Cor a 14 in northern Europe has been shown to identify ≥90% children with objective reactions to hazelnut.^{15,16,32}

A limitation of our study is the lack of data for Cor a 8 and other identified hazelnut allergen components. Cor a 8 is a common sensitizer in the Mediterranean area, although LTP sensitization is also increasingly demonstrated in non-Mediterranean areas, but its clinical relevance is unclear.^{29,46,47} In the only eligible study from southern Europe included in this study, Cor a 8 sIgE levels in children were low and did not differ between allergic and tolerant subjects.³¹ Moreover, the two studies with data on Cor a 8 from northern/central Europe enrolled mainly adults.^{17,29} Specific IgE to Cor a 11, a vicilin-like storage protein from hazelnut, likely contributes to the clinical manifestations of hazelnut allergy in young children, although there are currently little available data.^{13,14}

A general strength of meta-analyses is the large number of subjects included, and our data are based on 635 children with suspected hazelnut allergy. Diagnosis of hazelnut allergy was defined by the outcome of DBPCFC or open OFC, with the majority of studies using DBPCFC. By including studies with some patients diagnosed based on recent anaphylactic events, we also ensured representation of severe cases of hazelnut allergy. All pediatric studies were cross-sectional designs, resembling the clinical situation and minimizing the risk of overestimating diagnostic parameters.⁴⁸ This meta-analysis is limited by the small number of articles retrieved, but the datasets still enabled a reliable meta-analysis with low heterogeneity.

In conclusion, through a systematic approach and meta-analysis, we demonstrate that sIgE to hazelnut storage proteins Cor a 14 and Cor a 9 confers specificity in the diagnosis of hazelnut allergy in children. Using hazelnut extract to rule in IgE-mediated allergy in combination with sIgE to hazelnut storage proteins, children with primary hazelnut allergy can be more accurately identified, which would lead to a reduction in over-diagnosis and in OFC needed for a final diagnosis. Future analyses of the contribution of additional

hazelnut allergen components are needed, as well as evaluations in adult populations.

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CONFLICT OF INTEREST

Drs Berthold, Mascialino, and Sjölander are employees of Thermo Fisher Scientific. Dr Orme has received consultation fees from Thermo Fisher Scientific. The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

CN, MB, BM, SS, and RH contributed to the conception and design of the study. MB, BM, MO, and SS contributed to acquisition of data. MO performed the systematic literature search and data extraction, and BM performed the meta-analyses. All authors contributed to analysis and interpretation of data and were involved in drafting and revising the manuscript. All authors approved the final version for publication.

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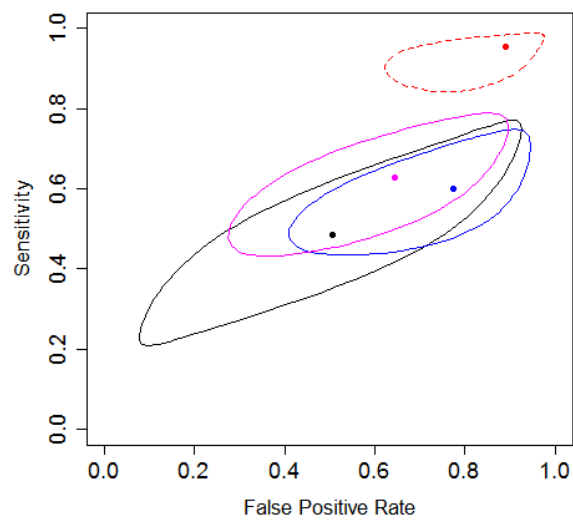
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SUPPORTING INFORMATION

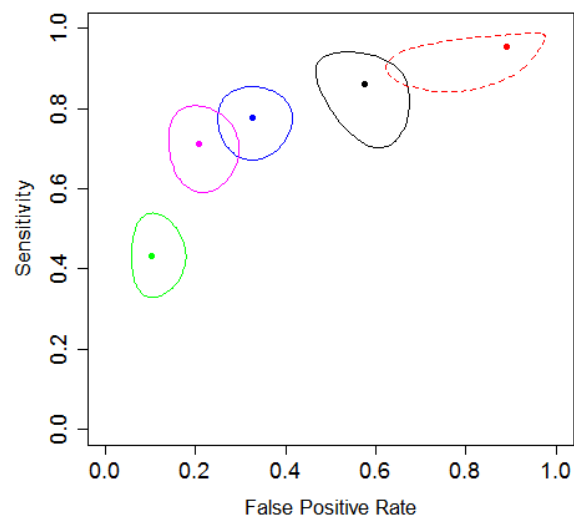
Additional supporting information may be found online in the Supporting Information section.

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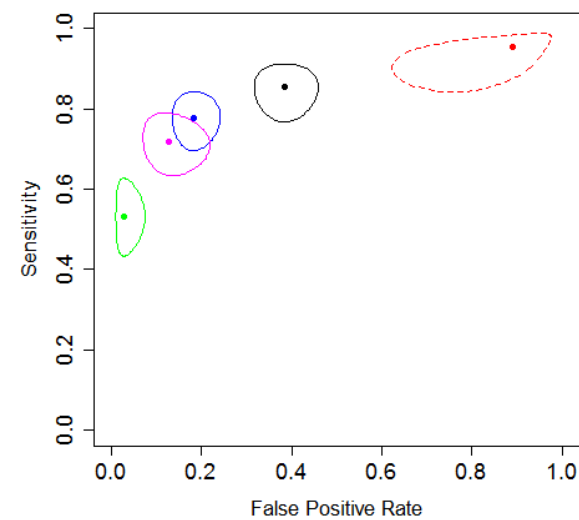
Cor a 1



Cor a 9



Cor a 14



0.1 kU_A/L

0.35 kU_A/L

1.0 kU_A/L

5.0 kU_A/L

f17 at 0.35 kU_A/L

eTable 1. Extracted data and associated sensitivity and specificity

From all eligible studies (0.35 kU_A/L cut-off); [†] identifies studies on adults (0.35 kU_A/L cut-off)

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Cor a 1	Brandström 2015	4	4	28	4	50.0 (15.7, 84.3)	12.5 (3.5, 29.0)
	Datema 2015 [†]	79	18	11	7	81.4 (72.3, 88.6)	38.9 (17.3, 64.3)
	Eller 2016	32	33	37	53	49.2 (36.6, 61.9)	58.9 (48.0, 69.2)
	Masthoff 2013 (children)	28	12	37	4	70.0 (53.5, 83.4)	9.8 (2.7, 23.1)
	Masthoff 2013 (adults) [†]	35	4	39	2	89.8 (75.8, 97.1)	4.9 (0.6, 16.5)
Cor a 8	Datema 2015 [†]	12	85	1	17	12.4	94.4

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
						(6.6, 20.6)	(72.7, 99.9)
	Masthoff 2013 (children)	3	37	2	39	5.0 (0.6, 16.9)	95.1 (83.5, 99.4)
	Masthoff 2013 (adults) [†]	2	37	1	40	5.1 (0.6, 17.3)	97.6 (87.1, 99.9)
Cor a 9	Brandström 2015	8	0	14	18	100.0 (63.1, 100.0)	56.3 (37.7, 73.6)
	Datema 2015 [†]	19	87	3	15	10.3 (5.1, 18.1)	83.3 (58.6, 96.4)
	Eller 2016	48	17	29	61	74.2 (61.5, 84.0)	67.9 (57.1, 77.3)
	Kattan 2014	11	2	10	19	84.6 (54.6, 98.1)	65.5 (45.7, 82.1)

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
	Masthoff 2013 (children)	33	7	8	33	83.0 (67.2, 92.7)	80.0 (65.1, 91.2)
	Masthoff 2013 (adults) [†]	14	25	2	39	36.0 (21.2, 52.8)	95.0 (83.5, 99.4)
Cor a 14	Beyer 2015	37	7	19	80	84.1 (69.9, 93.4)	80.8 (71.7, 88.0)
	Brandström 2015	8	0	5	27	100.0 (63.1, 100.0)	84.6 (67.2, 94.7)
	Buyuktiryaki 2016	22	4	3	22	84.6 (65.1, 95.6)	88.0 (68.8, 97.5)
	Datema 2015 [†]	10	79	0	17	11.2 (5.5, 19.7)	100.0 (80.5, 100.0)
	Eller 2016	52	13	14	76	80.0	84.4

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
						(68.2, 88.9)	(75.3, 91.2)
	Kattan 2014	9	4	5	24	69.2 (38.6, 90.9)	82.8 (64.2, 94.2)
	Masthoff 2013 (children)	28	12	10	31	70.0 (53.5, 83.4)	75.6 (59.7, 87.6)
	Masthoff 2013 (adults) [†]	15	24	2	39	38.0 (23.4, 55.4)	95.0 (83.5, 99.4)
Hazelnut sIgE	Beyer 2015	43	1	78	21	98.0 (88.0, 99.9)	21.0 (13.6, 30.6)
	Brandström 2015	8	0	31	1	100.0 (63.1, 100.0)	3.1 (0.1, 16.2)
	Buyuktiryaki 2016	24	2	20	5	90.6 (74.9, 99.1)	21.9 (6.8, 40.1)

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
	Masthoff 2013 (children)	40	0	41	0	100.0 (91.2, 100.0)	0.0 (0.0, 8.6)
	Masthoff 2013 (adults) [†]	39	0	41	0	100.0 (91.0, 100.0)	0.0 (0.0, 8.6)

From studies in children (0.10 kU_A/L cut-off), all study designs

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
Cor a 1	Brandström 2015	4	4	29	3	50.0 (15.7, 84.3)	9.4 (2.0, 25.0)
	Buyuktiryaki 2016	4	25	0	26	13.8 (3.9, 31.7)	100.0 (86.8, 100.0)
	Eller 2016	32	33	45	45	49.2 (36.6, 61.9)	50.0 (39.3, 60.7)
	Kattan 2014	11	2	23	6	84.6 (54.6, 98.1)	20.7 (8.0, 39.7)
Cor a 8	Buyuktiryaki 2016	12	16	11	16	42.9 (24.5, 62.8)	59.3 (38.8, 77.6)
	Kattan 2014	6	7	6	23	42.6 (19.2, 74.9)	79.3 (60.3, 92.0)
Cor a 9	Brandström 2015	8	0	16	16	100.0	50.0

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
						(63.1, 100.0)	(31.9, 68.1)
	Buyuktiryaki 2016	19	7	18	7	73.1 (52.2, 88.4)	28.0 (12.1, 49.4)
	Eller 2016	59	6	50	40	90.3 (81.0, 96.5)	44.4 (34.0, 55.3)
	Kattan 2014	12	1	16	13	92.3 (64.0, 99.8)	44.8 (26.5, 64.3)
Cor a 14	Beyer 2015	40	4	40	59	92.0 (78.3, 97.5)	60.0 (49.3, 69.3)
	Brandström 2015	8	0	9	23	100.0 (63.1, 100.0)	72.0 (53.3, 86.3)
	Buyuktiryaki 2016	25	1	9	16	95.5 (80.4, 99.9)	64.0 (42.5, 82.0)
	Eller 2016	53	12	37	53	81.5	58.6

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
						(70.0, 90.1)	(48.0, 69.2)
	Kattan 2014	11	2	10	19	84.6 (54.6, 98.1)	65.5 (45.7, 82.1)
Hazelnut sIgE	Beyer 2015	44	0	88	11	99.0 (92.0, 100.0)	11.0 (5.7, 19.0)
	Brandström 2015	8	0	31	1	100.0 (63.1, 100.0)	3.1 (0.1, 16.2)
	Carraro 2016	24	0	12	0	100 (85.8, 100.0)	0.0 (0.0, 26.5)
	Eller 2016	65	0	20	70	100.0 (94.5, 100.0)	77.8 (67.8, 85.9)
	Kattan 2014	13	0	29	0	100.0 (75.3, 100.0)	0.0 (0.0, 11.9)

From studies in children (1.0 kU_A/L cut-off), all study designs

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
Cor a 1	Brandström 2015	4	4	29	3	50.0 (15.7, 84.3)	9.4 (2.0, 25.0)
	Eller 2016	31	34	29	61	47.7 (35.2, 60.5)	67.8 (57.1, 77.3)
	Masthoff 2013	24	16	34	7	60.0 (43.3, 75.1)	17.1 (7.2, 32.1)
Cor a 9	Brandström 2015	7	1	8	24	87.5 (43.4, 99.7)	75.0 (56.6, 88.5)
	Carraro 2016	18	6	3	9	75.0 (53.3, 90.2)	75.0 (42.8, 94.5)
	Eller 2016	42	23	21	69	64.5 (51.8, 76.1)	76.5 (66.6, 84.9)
	Kattan 2014	11	2	6	23	84.6	79.3

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
						(54.6, 98.1)	(60.3, 92.0)
	Masthoff 2013	30	10	2	39	75.0 (58.8, 87.3)	95.0 (83.5, 99.4)
Cor a 14	Beyer 2015	33	11	9	90	74.0 (59.7, 86.8)	91.0 (83.4, 95.8)
	Brandström 2015	7	1	2	30	87.5 (47.4, 99.7)	94.0 (79.2, 99.2)
	Buyuktiryaki 2016	19	7	0	25	72.7 (52.2, 88.4)	100.0 (86.3, 100.0)
	Eller 2016	46	19	14	76	70.8 (58.2, 81.4)	84.2 (75.4, 91.2)
	Kattan 2014	9	4	0	29	69.2 (38.6, 90.9)	100.0 (88.1, 100.0)
	Masthoff 2013	28	12	10	31	70.0	76.0

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
						(53.5, 83.4)	(59.7, 87.6)
Hazelnut sIgE	Beyer 2015	41	3	65	34	94.0 (81.3, 98.6)	34.0 (25.1, 44.6)
	Brandström 2015	7	1	27	5	87.5 (47.4, 99.7)	15.6 (5.3, 32.8)
	Eller 2016	49	16	43	47	75.4 (63.1, 85.2)	52.2 (41.4, 62.9)
	Masthoff 2013	40	0	39	2	100.0 (91.2, 100.0)	4.9 (0.6, 16.5)

From studies in children (5.0 kU_A/L cut-off), all cross-sectional studies

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
Cor a 9	Brandström 2015	3	5	5	27	37.5 (8.5, 75.5)	84.4 (67.2, 94.7)
	Eller 2016	25	40	9	81	38.7 (26.7, 51.4)	90.1 (81.9, 95.3)
	Kattan 2014	7	6	0	29	53.8 (25.1, 80.8)	100.0 (88.1, 100.0)
	Masthoff 2013	19	21	1	40	48.0 (31.5, 63.9)	98.0 (87.1, 99.9)
Cor a 14	Brandström 2015	4	4	1	31	50.0 (15.7, 84.3)	96.9 (83.8, 99.9)
	Buyuktiryaki 2016	12	14	0	25	45.5 (26.6, 66.6)	100.0 (86.3, 100.0)
	Eller 2016	36	29	1	89	55.4	98.9

Index test	Reference	True Positives	False Negatives	False Positives	True Negatives	Sensitivity (%)	Specificity (%)
						(42.5, 67.7)	(94.0, 100.0)
	Kattan 2014	5	8	0	29	38.5 (13.9, 68.4)	100.0 (88.1, 100.0)
	Masthoff 2013	24	16	1	40	60.0 (43.3, 75.1)	97.6 (87.1, 99.9)
Hazelnut sIgE	Brandström 2015	7	1	20	12	87.5 (47.4, 99.7)	37.5 (21.1, 56.3)
	Buyuktiryaki 2016	16	16	1	31	50.0 (31.9, 68.1)	96.9 (83.8, 99.9)
	Masthoff 2013	33	7	27	14	82.5 (67.2, 92.7)	34.2 (20.1, 50.6)

eTable 2. Quality assessment of included studies

Coding for judgment	Representative spectrum	Acceptable reference standard	Acceptable delay between tests	Partial verification avoided	Differential verification avoided	Incorporation avoided	Index test results blinded	Reference standard results blinded	Relevant clinical information	Uninterpretable results explained	Missing data explained	Cut-off values pre-specified	Sponsoring precluded
Beyer 2015	yes	yes	yes	yes	no	yes	yes	unclear	yes	yes	yes	yes	no
Brandström 2015	yes	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	no	no
Buyuktiryaki 2016	yes	yes	yes	yes	no	yes	yes	unclear	yes	yes	no	yes	yes
Carraro 2016	yes	no	unclear	yes	no	yes	unclear	unclear	yes	yes	yes	no	unclear
Datema 2015	yes	no	yes	yes	no	yes	yes	yes	yes	yes	no	yes	yes
Eller 2016	yes	yes	no	yes	no	yes	yes	unclear	yes	yes	yes	no	no
Kattan 2014	no	no	no	yes	no	yes	yes	unclear	unclear	yes	yes	no	yes
Masthoff 2013	no	no	yes	yes	no	yes	yes	unclear	yes	yes	yes	yes	no

- Representative spectrum: Two studies excluded patients and may not be representative of patients who will receive the test in clinical practice (Kattan, 2014; Masthoff, 2013).
- Acceptable reference standard: Four studies were marked down because a positive OFC challenge could have been determined by subjective clinical symptoms, or diagnosis of hazelnut allergy was based on subjective clinical evidence of allergy other than anaphylaxis (Carraro, 2016; Datema, 2015; Kattan, 2014; Masthoff, 2013).
- Acceptable delay between tests: Two studies were marked down because hazelnut allergy was based on clinical history or previous anaphylaxis made more than six months prior to study enrolment (Eller, 2016; Kattan, 2014).
- Differential verification avoided: Apart from Brandström *et al.*, all studies were marked down because not all patients received the same reference standard. Some patients were classified based on OFC and others based on clinical history or previous anaphylaxis. This does not imply that the methodological quality of these studies was low; differential verification of disease status is standard practice since OFC would not be appropriate for all patients.
- Reference standard results blinded: One study was marked down because the index test results were not interpreted without knowledge of the results of the reference standard (Brandström, 2015).
- Missing data explained: Two studies did not explain missing component test results (Buyuktiryaki, 2016; Datema, 2015).
- Cut-off values pre-specified: Four studies did not pre-specify cut-off values for assessing sensitivity and specificity of the index test prior to starting the study (Brandström, 2015; Carraro, 2016; Eller, 2016; Kattan, 2014).