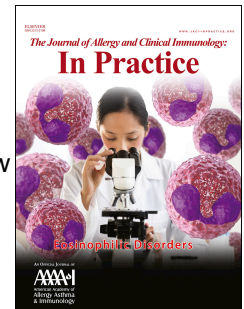


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The accuracy of diagnostic testing in determining tree nut allergy: a systematic review

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TITLE

The accuracy of diagnostic testing in determining tree nut allergy: a systematic review

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABSTRACT**Background**

Food allergy is most accurately diagnosed by a formal oral food challenge however it is time and labor intensive, risks the individual to severe reaction and access is often a limiting step in the diagnostic process. This is compounded for tree nut allergy diagnosis as several oral food challenges may be required to determine allergy status to each individual tree nut. Accurate diagnosis using minimally invasive diagnostic tests in predicting clinical tree nut allergy is important to correctly identify those with potentially life-threatening reactions, and to efficiently and safely tailor nut avoidance to only those nuts deemed allergic to enable less restricted diets and increased food choices for food allergic individuals.

Objective

To conduct a systematic review on the diagnostic capacity of clinical tests (Skin Prick Test (SPT), specific IgE, Component Resolved Diagnostics (CRD), Basophil Activation Test (BAT)) to determine oral food challenge proven or clinical tree nut allergy.

Methods

We searched four electronic databases (OVID Medline, Embase, Cochrane library and PubMed) until May 2020. Eligible studies were categorized by type of tree nut, and diagnostic test.

Results

27 studies assessed diagnostic accuracy to a specific tree nut. Overall, the accuracy of diagnostic testing was only reasonable, with 95% positive predictive values established in a small number of tree nuts. Cashew has best diagnostic accuracy, with the cashew component Ana o 3 being most predictive. At the other end of the spectrum, diagnostic testing of almond is poor and of limited clinical use.

Conclusion

The systematic review highlights the limitations of our current diagnostic tools for tree nut allergy and highlights further areas for research. The uni-directionality of cross-reactivity between cashew/pistachio and walnut/pecan is described and can aid diagnosis. Using diagnostic algorithms such as those demonstrated for walnut/pecan and cashew/pistachio allergy may achieve greater diagnostic accuracy and reduced number of OFCs.

Highlights

What is already known about this topic?

- Sensitization to tree nuts is much greater than the rates of true clinical tree nut allergy. This may lead to increased oral food challenges (OFC), which are often a limiting step in the diagnostic process.

What does this article add to our knowledge?

- This study describes the diagnostic capacity of current clinical tests in determining tree nut allergy. 95% PPVs are identified in cashew and walnut, however many tree nuts have limited diagnostic capacity and do not reduce need for OFC.

How does this study impact current management guidelines?

- This systematic review outlines the current strengths and limitations of the available diagnostic tests for tree nuts. This data may be used as a clinician reference when deciding on diagnostic testing in a clinical setting.

Key Words

Tree nut allergy, food allergy, diagnostic accuracy, diagnosis, positive predictive value, sensitivity, specificity, area under the curve, skin prick test, IgE, component resolved diagnostics, basophil activation test, cashew, pistachio, walnut, pecan, hazelnut, almond, brazil nut, macadamia.

ABBREVIATIONS

SPT	Skin prick test
sIgE	Specific Immunoglobulin E
CRD	Component Resolved Diagnostics
PPV	Positive predictive value
OFC	Oral food challenge
NPV	Negative predictive value
BAT	Basophil activation test
DBPCFC	Double-blinded, placebo-controlled oral food challenge
AUC	Area under the curve

INTRODUCTION

'Tree nuts' are a collective term referring to a group of nuts, seeds and fruits which grow on trees. These include almond, cashew, hazelnut, pistachio, walnut, pecan, macadamia, and Brazil nut. The prevalence of individual tree nut allergies varies throughout the world, with cashew the most prevalent in Australia(1), hazelnut in Europe(2), walnut and cashew in the United States(3) and Brazil nut, almond and walnut in the United Kingdom(4, 5). The reasons for this may include geographic variants, diet exposure effects, age, gender, race and ethnicity differences as well as multiple other factors(6).

Tree nut sensitization ranges from 0.8%-12.2%, significantly more than those with a true clinical allergy, estimated at less than 2% for oral food challenge (OFC) confirmed tree nut allergy(6, 7). Peanut and tree nut allergy have been identified as the main culprits of fatal or near-fatal reactions in children(8, 9). Tree nut allergies account for 18-40% of reported food induced fatalities(10-12). Most of these children have had a reaction to more than one nut, with around 20-30% of people with a peanut allergy also allergic to one or more tree nuts(7). Tree nut allergy constitutes a life-long risk for many allergic individuals given its low rate of resolution (~9%) (13).

Diagnosis of tree nut allergy

A fundamental concept in the diagnosis of food allergy is the understanding that false positive results of sensitization are not infrequent, and 'screening' can result in a high rate of misdiagnosis. Traditionally, many individuals who are peanut or tree-nut allergic are sensitized (i.e. screened on skin prick test (SPT) to other tree nuts but

have never had oral allergen exposure). A major challenge in managing individuals with suspected tree nut allergy is to distinguish between sensitization and allergy(14).

The diagnostic gold-standard of food allergy is to perform an OFC, however this is time and labor intensive, limited in availability in many tertiary allergy services, and exposes the child to the risk of severe reactions(15). In addition to skin prick tests (SPT), in vitro assays such as the measurement of circulating food specific IgE (sIgE) antibodies and basophil activation tests (BAT) have aided in the diagnosis of food allergy. One application of these tests is to identify a threshold with 95% positive predictive values (PPV), that is, a result equal or greater than the threshold is associated with a 95% probability of being allergic. However, there is often a low sensitivity, therefore a substantial proportion of individuals cannot have an accurate diagnosis because they fall into the diagnostic 'grey zone' of a positive skin prick test but below this threshold (15, 16). Component resolved diagnostics (CRD) is the measurement of individual components of the single food sIgE allergen, has helped improve the diagnosis of some food allergies such as peanut(16) however this has not been evaluated in detail for tree nuts.

Diagnosis of food allergy is not generally based on a single test. A stepped approach can be used, firstly with clinical history determining foods to be tested, and the result of that test (i.e. usually a test of sensitization such as SPT, or IgE measurement) can be used to determine whether an OFC is warranted. For example, in peanut allergy, using Ara h2 in a step wise approach (i.e. SPT, followed by Ara h 2 if SPT 3-8mm) would result in a 2.5-4 times reduction in number of required supervised OFCs to establish an accurate diagnosis(16).

Tree nut allergy diagnosis is problematic as many individuals may be sensitized to multiple nuts without a history of prior reaction or oral ingestion. Many clinicians may err on the side of caution and accept a diagnosis of 'possible' food allergy based on sensitization status alone(16). There is now evidence that avoidance of foods increases the risk of allergy with time so this practice warrants review(1).

Objectives

The objectives of this systematic review are to assess and present the published data on the diagnostic capacity of multiple clinical tests to determine true OFC proven allergy.

METHODS

Search Strategy

This systemic review was conducted using the methods and procedures of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines(17). Systematic database searches were performed of four databases (OVID Medline, Embase, Cochrane library and PubMed). The search strategy was constructed with the assistance of a research librarian. The search strategy was created first in OVID Medline, then modified for Embase, PubMed and Cochrane library searches. The full OVID Medline search strategy is outlined in supplemental figure E1. Search strategy was executed on 25th February 2020, with further searches continued until 5th May 2020 to ensure most recent studies were included.

Study Selection

Tree nuts were defined as walnut, almond, pistachio, cashew, pecan, hazelnut, macadamia and Brazil nut. Pine nut and sesame were not included in this review. Studies reporting a sensitivity, specificity, PPV or negative predictive value (NPV) for diagnostic test cut-offs were included and presented. No age restrictions were applied. Diagnostic tests included SPT, serum specific IgE to whole nuts, serum specific IgE to nut components and basophil activation tests (BAT). We included studies that showed a clinical outcome of tolerance or allergy either by double-blinded placebo-controlled food challenge (DBPCFC), open food challenge, or recent history of objective allergic reaction or anaphylaxis within a recent time frame (2 years). The search included population-based, and outpatient clinic-based groups. The search was limited to English-language articles. We excluded studies that defined allergy as sensitization, where the individual had a positive test but no clear history of ingestion and reaction. Studies were also excluded if the tree nut data was presented as a unifying group ('tree nuts'), rather than individual tree nut. Case reports, reviews and studies without full-text articles were excluded.

Identified articles were screened via title and abstract by two independent reviewers (TB, TD). Discrepancies were resolved by consensus. Reference lists of identified studies were reviewed for additional articles. Full text review was then performed by the two independent reviewers for the identified articles, and those meeting inclusion criteria were included, with discrepancies resolved by consensus. Data was extracted by the 1st author. The full search method is summarized below in figure 1.

Analysis

Studies were grouped according to tree nut and relevant study details were summarized including reference details, country and recruitment of cohort, age, sample size and allergy rates and determination method for allergy. For SPT, choice of allergen (commercial extract or fresh tree nut) was described for each study. Cashew and pistachio, and walnut and pecan were grouped together given the high correlation of co-existent allergy (18). For each diagnostic test, sensitivity, specificity, PPVs and NPVs and Area under the curve (AUC) were extracted. In studies where multiple cut-offs were reported, the optimal cut-off as reported by the study were included. In the case of nut component IgEs where multiple components outcomes were reported, the component or components that were identified by the study to be most accurate in diagnosing true allergy were included. Criteria assessing risk of bias and applicability was assessed according to the QUADAS-2 tool(19) and presented in Table I.

For this review, the study populations in each study were grouped as follows:

1. Outpatient: defined as a patient referred to a tertiary hospital allergy outpatient service with concern regarding allergy to food. This includes both those specific to the tree nut tested, and those not specific. All patients with positive SPT proceeded to OFC. These were prospective studies.
2. Population cohort: defined as a sample of participants recruited from the general population, inclusive of those with and without food allergy concerns.
3. Retrospective: defined as a patient seen by a clinical allergist/physician and referred for an OFC to define their allergen status. Data was collected retrospectively. This group does not include all patients with a positive SPT, which may give a selection bias to this population.

For this review, the approach confirming a tree nut allergy (reference standard) was grouped as either DBPCFC, open OFC or clinical history, for which the definitions are summarized in supplemental table E1.

RESULTS

The search methodology is summarized in figure 1. The search identified 1354 articles. Of these, 27 met the inclusion criteria and were included in this systematic review.

The included studies are described in tables II to VI below.

Cashew/Pistachio (Tables IIa-d)

SPT (Table IIa)

5 studies were identified, of which 3 studies were of high quality(14, 15, 20). Study size ranged from 61 to 531. One study included a large population cohort (15) while the others were classified as outpatient (n=3) or retrospective (n=1).

Clinical accuracy of skin prick tests to determine cashew and pistachio allergy are good, with the AUCs presented ranging between 0.81-0.94 for cashew, and pistachio 0.85-0.89(14, 15, 20).

McWilliam and Elizur report a 95% PPV for cashew in a clinic cohort of ≥ 14 mm and ≥ 12 mm respectively(14, 15). Cetinkaya et al reported a lower optimal SPT size of ≥ 6.25 mm but did not report a 95% PPV(20). McWilliam also reported a population cohort, with 95% PPV of 10mm (95% CI 7.5-12mm). Ho et al reported SPT ≥ 8 mm to have 100% PPV to cashew allergy in a retrospective study (21). Using these cut-offs, sensitivity in the population or outpatient cohorts ranged between 0.42-0.92. Specificity ranged between 0.79-0.98. NPVs were also presented, ranging between 0.48-0.97(14, 15, 20).

Two of the three studies used a fresh cashew extract, and one a commercial extract. McWilliam' s study used Hollister-Stier in their clinician cohort, and ALK (Spain) for their population cohort. Elizur made a fresh cashew extract consisting of cashew flour mixed with water to a dilution of 10mg/mL. Cetinkaya also used a fresh cashew extract using defatted raw cashew powder extracted using a buffer solution, of which the supernatant was extracted to use as an extract.

SPT to pistachio is limited to three studies, for which a 95% PPV has not been established(14, 20, 21). The best sensitivity and specificity are demonstrated in Elizur' s study, with ≥ 7 mm giving optimal sensitivity and specificity (0.92 and 0.77 respectively)(14). Cetinkaya found a similar optimal SPT at ≥ 7.25 mm but with lower sensitivity and specificity (0.82 and 0.75 respectively). NPV was reported in this study at 0.92 for the cut-off of ≥ 7.25 mm(20).

Elizur again used a fresh pistachio extract, made by grinding pistachio to a paste and mixing with water to a dilution of 10mg/mL. Cetinkaya used the same process

described above with cashew. Ho' s study used commercial extract, Dome-Hollister-Stier.

Cashew and Pistachio IgE (whole nut) (Table IIb)

3 studies were identified(20, 22, 23), with two studies assessed as high quality(20, 22). All studies were classified as outpatient studies.

Specific IgE to cashew or pistachio (whole nut IgE) appears a similar diagnostic tool than SPT, but AUC is still good, ranging between 0.79-0.89(20, 22, 23). Reasonable sensitivity but poor specificity are reported by all studies (sensitivity 0.81-1.0, specificity 0.54-0.75) with broad optimal cut-off values ranging between ≥ 0.35 - ≥ 2.62 kUA/L(20, 22, 23). To date a 95% PPV has not been reported for specific IgE to cashew or pistachio. NPV was reported in Cetinkaya' s study as 1.0 using the cut-off ≥ 1.125 kUA/L, however this population included only 13 cashew allergic patients(20).

IgE to Cashew components (Table IIc)

Three studies report on the diagnostic accuracy of Ana o 3 in diagnosing cashew allergy, all outpatient studies. AUC for Ana o 3 is much greater than SPT or whole nut IgE(22-24), ranging between 0.92 and 0.98. Using Ana o 3 as a diagnostic tool in one study for pistachio allergy reported AUC of 0.97(23).

Lange et al, is the only study to produce a 95% PPV of 2.0kUA/L. Optimal sensitivity (0.93) and specificity (0.90) were found at ≥ 0.3 kUA/L, which is similar to the optimal cut-off of ≥ 0.35 kUA/L identified by Savatiano et al (sensitivity 0.98, specificity 0.94). Both studies were based on reasonable sized cohorts (Lange n= 61, Savatiano n=53)(23, 24). Savatianos' paper reported high sensitivity and specificity of 96.8% and 94.4% for pistachio allergy respectively when using Ana o 3 as a diagnostic tool(23). In a Japanese study of patients with a history of recent reaction to cashew (n=95), optimal sensitivity (0.85) and specificity (0.88) was reported at a higher Ana o 3 cutoff (≥ 0.70 kUA/L)(22). NPV was not described in these studies.

Basophil activation test (BAT) to Cashew and Pistachio (Table IId)

Only one study was identified that examined the predictive value of BAT for diagnosing cashew and pistachio allergy (14) reporting a 95% PPV of $\geq 5.5\%$ CD63 activation to cashew. Optimal sensitivity (0.77) and specificity (0.93) to cashew were

found at $\geq 2\%$ CD63+ activation and pistachio at $\geq 5.8\%$ (sensitivity 0.77, specificity 0.88). NPV was not reported. This study reports no false negative results using an algorithm combining inconclusive-positive SPT ($3\text{mm} \leq \text{SPT} \leq 12\text{mm}$) and BAT(14).

Walnut/Pecan (Tables IIIa-d)

SPT (Table IIIa)

Three studies were identified, two studies were of high quality(14, 25). Study size ranged from 29 to 71. Two studies were classified as outpatient, and one was retrospective.

Clinical accuracy of SPT to determine walnut and pecan were described in two studies, Blankestijn et al reporting an AUC of 0.80 for Walnut (25) and Elizur et al an AUC of 0.89 for walnut and 0.84 for Pecan(14).

All three studies reported a 95% PPV or greater with SPT result $\geq 8\text{mm}$ (14, 21, 25). Of the two higher quality studies, optimal sensitivity and specificity was reported at a lower threshold, with Elizur reporting sensitivity of 0.87, specificity 0.79 using SPT result $\geq 6\text{mm}$ (14). Similarly, Blankestijn's study reported SPT result $\geq 5\text{mm}$ giving a sensitivity of 0.55, and specificity 0.95. NPV was reported in this study at 0.58 using SPT $\geq 5\text{mm}$, and 0.48 using SPT $\geq 8\text{mm}$ (25).

Blankestijn and Ho's studies both used a commercial walnut extract (ALK1 and Dome-Hollister-Stier respectively) for their SPTs(25). Elizur used a fresh walnut extract, created by use of walnut flour mixed with water to a dilution of 10mg/mL(14).

Pecan is described in only one high-quality study, with SPT cut-off of 5mm producing an AUC of 0.84 but reporting no PPV(14). The best sensitivity (0.83) and specificity (0.79) were found in Elizur's study at a cut-off of $\geq 5\text{mm}$ (14). Ho et al's study found a higher optimal cut-off of $\geq 7\text{mm}$, with higher specificity (0.96), but lower sensitivity (0.5)(21).

Elizur used a fresh Pecan extract, made by grinding fresh pecan to a paste and diluting with water to achieve a concentration of 10mg/mL. Ho's study used a commercial extract, Dome-Hollister-Stier(14, 21).

Walnut IgE (whole nut) (Table IIIb)

Three studies are identified, all classified as high quality. Study size ranged from 55 to 108.

IgE to walnut AUC appears similar to that of SPT ranging from 0.79 to 0.86, however there is a large range in optimal cut-offs reported.

95% PPV is reported in one study by Elizur et al, with optimal cut-off $\geq 5.07 \text{ kUA/L}$ (13). Regarding optimal cut-offs for sensitivity and specificity, thresholds ranged from $\geq 0.46 \text{ kUA/L}$ to $> 2.58 \text{ kUA/L}$. Sensitivity ranges reported at these thresholds was 0.62-0.73, with specificity 0.70-0.91. NPV ranged between 0.36-0.67 (13, 25, 26).

There were no studies that reported data for Pecan whole nut IgE.

IgE to Walnut components (Table IIIc)

The same three studies describing IgE to whole walnut also described IgE to walnut components (CRD) (13, 25, 26). Jug r 1 had a similar AUC to whole nut IgE, ranging from 0.80-0.85. Only one study reported a 95% PPV of 0.21 kUA/L (13). Optimal cut-offs reported ranged between $\geq 0.1 \text{ kUA/L}$ to $\geq 0.423 \text{ kUA/L}$, with similar sensitivity (0.61-0.87) and specificity (0.79-0.91). NPV was reported in all three studies, ranging between 0.6-0.81 (13, 25, 26).

No studies report data for Pecan component IgE.

Basophil activation test (BAT) to Walnut and Pecan (Table IIIId)

Only one study has investigated the diagnostic accuracy of BAT to walnut and pecan. This study of 71 patients identified a 95% PPV to walnut allergy of $\geq 6.4\%$ CD63+ activation, with sensitivity of 0.8, specificity 0.88. Pecan allergy sensitivity and specificity was optimal at $\geq 2.7\%$ (0.74 and 0.83 respectively). NPV was not reported in this study. No false negative results were reported using an algorithm combining inconclusive-positive SPT ($3 \text{ mm} \leq \text{SPT} < 8 \text{ mm}$) and BAT (14).

Hazelnut (tables IVa-d)

SPT (Table IVa)

Five studies, two assessed as high quality, were identified reporting diagnostic accuracy of hazelnut SPT (14, 27). Study size ranged from 58-155. Three studies were classified as outpatient studies, and the remaining two were retrospective.

Diagnostic accuracy of hazelnut SPT is less than that of walnut, with AUC ranging between 0.75-0.80(14, 21, 27-29). Three studies reported similar SPT optimal cut-offs of $\geq 7\text{mm}$ - $\geq 8\text{mm}$, (14, 27, 28). Sensitivities and specificities were similar, ranging from 59-64% and 77-90% respectively.

No high quality studies identified a 95% PPV for hazelnut. Using the optimal SPT cut-offs of $\geq 7\text{mm}$ - $\geq 8\text{mm}$, PPV ranged between 0.38-0.91, with NPV 0.56-0.91(14, 27).

Two studies used fresh hazelnut alone as their allergen(14, 28), with two other studies using commercial extracts(21, 27), and one study using both commercial and fresh(29). Elizur used fresh hazelnut extract, made with hazelnut flour, diluted in water to a concentration of 10mg/mL(14), whereas Eller and Ortolani used a prick-to-prick method with the whole hazelnut(28, 29). Ortolani also used commercial extract with multiple sites using different extracts and pooling the results(29). Buyuktiryaki used Alyostal hazelnut extract, and Ho used Dome-Hollister-Stier extract(21, 27).

Hazelnut IgE (whole nut) (Table IVb)

Five studies were identified of which two were of high quality(27, 30). Study size ranged from 36-151. Four studies were classified as outpatient, and one retrospective.

The AUC for hazelnut IgE is similar to SPT, ranging from 0.71-0.87, however the range of optimal cut-off is wide (0.35-7.25kUA/L) in the five studies(27, 29-32). This makes it difficult to define a clinical decision point cut-off.

There is no documented 95% PPV for IgE to hazelnut. The highest sensitivity and specificity were reported by Buyuktiryaki, who report an optimal cut-off of $\geq 3.15\text{kUA/L}$ (sensitivity 71%, specificity 91%)(27). Ortolani reports their optimal cut-off as $\geq 0.7\text{kUA/L}$ with sensitivity of 75%, but a poor specificity of 17%(29). NPV ranges between 0.05 in Ortolani's study using $\geq 0.7\text{kUA/L}$, to 0.97 in Beyer's study using $\geq 0.35\text{kUA/L}$ (29, 30). Buyuktiryaki's higher cutoff of $\geq 3.15\text{kUA.L}$ produced a NPV on 0.81(27).

IgE to Hazelnut Components (Table IVc)

Eight studies were identified of which four are considered of higher quality(27, 30, 33, 34). Study size ranged from 36-155. Four studies were classified as outpatient and the remaining four were retrospective.

Hazelnut components Cor a 9 and Cor a 14 provide greater diagnostic accuracy with higher AUC (0.71-0.98) compared to whole nut IgE and SPT. However there remains no clear consensus from multiple studies on the optimal cut-off for clinical diagnosis(27, 28, 30, 31, 33-35).

Variable optimal cut-offs to Cor a 14 have been reported, ranging from 0.63-5kUA/L(27, 28, 31, 33, 34). Using these cut-offs, sensitivity ranged between 0.60-1.0 and specificity between 0.92-1.0. Beyer reports on a traditional cut-off of ≥ 0.35 kUA/L, rather than an optimal level with sensitivity 0.85 and specificity 0.81(30).

Buyuktiryaki report a 100% PPV using a cut-off of ≥ 0.63 kUA/L, but no other studies reported a 95% PPV for hazelnut CRD. NPV for this cut-off was 0.86(27).

Basophil Activation Test (BAT) to Hazelnut (Table IVd)

Two studies, both of high quality were identified(14, 34). These two outpatient studies had 61 and 40 patients respectively.

Whilst limited to two studies, AUC is far greater for BAT compared to other diagnostic tools, ranging between 0.94 and 0.98. Elizur reports optimal sensitivity (1.0) and specificity (0.87) using a cutoff of $\geq 3.4\%$ CD34+ activation, although no 95% positive predictive value could be established(14). This compares to Brandstrom, who described better accuracy using a lower cutoff of $\geq 1.7\%$ (sensitivity 1.0, specificity 0.97), again with no false negative BAT results(34).

Almond (tables Va, Vb)

SPT (Table Va)

All three studies identified were of lower quality, using retrospective analysis of clinician referred OFCs to determine optimal cut-off(21, 36, 37). Study size ranged from 28 to 590.

Only one study reported AUC, which was poor (0.66)(37).

Variable optimal cutoffs were reported in two studies(21, 37), Virkud ($\geq 11\text{mm}$) and Ho ($\geq 7\text{mm}$). Both show poor sensitivity (0.33 and 0.14 respectively) (21, 37). Baker and colleagues did not present the data as an optimal cutoff, but showed a high negative OFC rate (tolerant), despite high SPT results (see table Va)(36). Only Ho's study reported the type of SPT allergen, using commercial extract (Dome-Hollister-Steir)(21).

IgE to Almond (whole nut) (Table Vb)

Two studies were identified evaluating the diagnostic accuracy of IgE to almond and these were the same studies reporting SPT outcomes for almond allergy described earlier(36, 37). Only one study(37) identified an optimal cut-off value of $\geq 10\text{kUA/L}$ with an AUC of 0.61 (36).

IgE to Almond components and Basophil Activation Test (BAT) to Almond

Whilst almond protein components have been identified(38), diagnostic ability has not been evaluated.

Brazil nut (Tables VIa-c)

SPT (Table VIa)

One retrospective study, of poor quality and limited to 10 participants was identified reporting an optimal cut-off of $\geq 8\text{mm}$ (21). Sensitivity and specificity were not reported, and AUC data is not presented for any Brazil nut diagnostic tools. This study used a commercial extract (Dome-Hollister-Steir)(21).

IgE to Brazil nut (whole nut) (Table VIb)

One study was identified reporting an optimal Brazil nut IgE cut-off of $\geq 1.51\text{kUA/L}$ (39), in an outpatient cohort, however sensitivity was poor (0.42). This study comprised 34 patients.

IgE to Brazil nut components (Table VIc)

One study, the same which reports on IgE to brazil nut, also reports an optimal cut-off of 0.25kUA/L for the Brazil nut component Ber e 1(39). This showed greater sensitivity and similar specificity compared to whole nut IgE, using the same cohort ($n=36$).

Basophil Activation Test (BAT) to Brazil nut

No studies were identified reporting on the diagnostic accuracy of BAT in Brazil nut.

Macadamia

No studies were identified assessing the accuracy of any diagnostic tests to macadamia.

DISCUSSION

This is the first systematic review reporting diagnostic test accuracy for all tree nuts across all age groups and different regions of the world.

This study looks in depth at the various diagnostic tests for tree nuts, both clinically and in a research setting. Generally speaking, SPTs outperform whole nut IgE in terms of diagnostic accuracy. 95% PPVs were reported for cashew and walnut, and hazelnut has been comprehensively studied, but few studies have reported thresholds that would reduce the need for OFCs to almond, macadamia, brazil nut, pistachio and pecan.

Reviewing all of the diagnostic tests for each tree nut, tests for cashew provide the greatest accuracy, with Ana o 3 in particular appearing to have high diagnostic value. There are defined positive predictive values for cashew allergy on skin prick testing, and the accuracy of cashew CRD (Ana o 3) is the highest of all component resolved diagnostics tests and appears the most accurate tool in predicting cashew allergy, assuming a positive skin prick test as this was the criteria for inclusion(24, 40). AUC for BAT to cashew is similar to that of CRD but appears less accurate for pistachio based on a single study.

There are no studies reporting the diagnostic accuracy of predicting pistachio allergy using IgE to pistachio components Pis v 1 or Pis v 2. These two components have been found in 68% and 50% respectively of pistachio allergic individuals(41). Savvatanos et al' s study demonstrating an AUC of 0.97 using Ana o 3 to predict pistachio allergy is an interesting outcome, given that cashew and pistachio cross reactivity is well demonstrated, but is not absolute, with pistachio allergy present in 65% of cashew allergic patients, and cashew allergy present in 100% of pistachio allergic patients in a pediatric population, median age 8.7 years of age(42). The rates of cross reactivity are not described in Savvatanos et al' s study.

The accuracy of the tools in diagnosis of cashew and pistachio allergy, specifically CRD and skin prick test would suggest that algorithms could be developed to predict

cashew and pistachio allergy with greater accuracy with a reduced need for OFC. In certain areas of the world, such as Australia where cashew allergy is the most prevalent tree nut allergy(43), Ana o 3 or its use in a clinical algorithm may be a useful diagnostic tool in future, similar to Ara h 2 in peanut allergy(16).

Walnut SPT accuracy is reasonable, with optimal skin prick test cut-offs of between 5-6mm(14, 25). Whole nut IgE and CRD optimal cut-off is not clearly defined with a wide range of cut-offs described. The range of results may suggest that either there is geographical variance, or that cohorts need to be larger to identify the true optimal cut-off. For walnut CRD, the component Jug r 1 is most accurate in predicting walnut allergy, however a 95% PPV was able to be calculated for the component Jug r 4 in Elizur et al' s study and appeared to be more predictive in diagnosis of dual walnut-pecan allergic patients(13). In line with other foods like peanut, Jug r 1, a 2S seed storage protein seems to be the dominant allergen in the three included from Dutch, Israeli, and Japanese cohorts, and provided the most accuracy for predicting allergy(13, 25, 26). Interestingly Elizur et al found that Jug r 4, an 11S protein, played a minor role in the Israeli cohort, with a sensitivity of only 31% compared to Jug r 1 with a sensitivity of 81%. A 95% PPV was able to be calculated for Jug r 4 and appeared more predictive in diagnosis of dual walnut-pecan allergic patients(13).

Pecan is poorly assessed across all diagnostic tests. Whilst there are no studies looking at Pecan CRD for diagnosis of Pecan allergy, Elizur et al' s study demonstrated an AUC of 0.77 using Walnut component Jug r 4 to predict pecan allergy. Of interest, there were no patients who were defined as 'pecan allergic, walnut tolerant' in the papers included in this study. However, a number of patients do have walnut allergy, despite tolerance to Pecan(14). This allergic unidirectionality may suggest that some components are shared by pecan and walnut, while others must be unique to walnut.

The uni-directionality of cross-reactivity has been previously described between cashew-pistachio and walnut-pecan in the ProNuts and Nut CRACKER studies(14, 44). Clinically, this can also aid in the diagnosis of allergy to these foods; patients with a negative OFC to cashew or walnut are unlikely to be allergic to pistachio or pecan, respectively, regardless of sensitization level. Elizur et al have proposed an order for OFC of these nuts, depending on whether there is a high or low probability of

reaction(45). They propose in patients suspected of high probability of walnut/pecan allergy based on clinical testing, Pecan be challenged first, noting that if positive, there is a high (near 100% chance) that the patient will also be walnut allergic. In patients with low probability for walnut/pecan allergy, then challenge firstly to walnut, noting if tolerant, the patient likelihood of pecan allergy is near zero. A similar algorithm applies for cashew and pistachio, enabling a significant reduction in need for OFC(45).

Hazelnut SPT optimal cut-off values were established at 7-8mm, however the AUC of these was lower (0.75-0.80) than found in other tree nuts, suggesting a sub-optimal diagnostic test used in isolation. For whole nut IgE or CRD, Hazelnut has a similar issue in that there is a wide range of reported optimal cut-off values and therefore overall is still a poor diagnostic tool in predicting allergy. While the majority of hazelnut studies reported the CRD for Cor a 9 and Cor a 14, only 4 reported PPVs(27, 28, 30, 34). Buyuktiyaki et al and Eller et al found Cor a 14 was the best performer for predicting hazelnut allergy, with thresholds of 0.63 and 0.72 reaching PPVs of 100% and 92% respectively(27, 28). Masthoff proposes that Cor a 9 and Cor a 14 be used in combination when determining allergic status to hazelnut(33).

In contrast, BAT is more accurate in diagnosis of hazelnut allergy, but there are limitations in its use in a clinical setting, outlined below. Given the range in optimal cut-off and the limited studies, further studies with larger patient numbers are likely required prior to establishing clear diagnostic cut-off values.

Nilsson et al(46) presented a systematic review and meta-analysis of hazelnut CRD, using a diagnostic cut-off of 0.35kUA/L for Cor a 9 and Cor a 14 and comparing to a cut-off of 0.1kUA/L and 1.0kUA/L. Cor a 14 and Cor a 9 appear the most accurate of the hazelnut components, with pooled data suggesting 0.35kUA/L produced the best sensitivity (65.1% and 64.6%) and specificity (84.9% and 74.1%), respectively(46). The data presented in our systematic review suggests similarly that Cor a 14 is slightly more accurate in diagnosis than Cor a 9, and these are the two major components in diagnostic testing for hazelnut allergy. Our study presents the data as optimal cut-off rather than at a set-value, and therefore the sensitivities and specificities are not directly comparable. 7 of the 8 studies included in Nilsson et al' s meta-analysis (46) were also included in our analysis, with one study not meeting the inclusion criteria.

The diagnostic tests used in assessing almond allergy appear poor and the prevalence of almond allergy is low (14). Baker et al chose not to present their data as an optimal cut-off for this reason(36). The majority of data suggests a high false-positive rate(36, 37). Given this, there is an argument that in a patient with no prior exposure, testing should not be done. The major rationale to perform diagnostic testing to almond would be in the setting of a suspected clinical reaction to determine whether to proceed with a supervised OFC, or recommend home introduction, rather than avoiding based on the outcome of the test.

Of the remaining tree nuts, Brazil nut has only limited data which is sub-optimal when using these tests as a diagnostic tool. Macadamia did not have any studies which assessed the accuracy of the diagnostic tests.

One important aspect to consider when looking to establish diagnostic thresholds is the reference standard. For those studies using a retrospective analysis of clinician-referred OFC, the analysis is of a subgroup of patients, which will falsely decrease sensitivity and increase specificity due to reduction in challenges for those with positive diagnostic tests (i.e. a high skin prick test is less likely to proceed to oral food challenge). Population-based studies where all individuals proceed to an OFC, or prospective studies where all patients with positive SPTs proceed to OFC are likely to produce the most accurate results. Similarly, regional prevalence differences may impact the clinical use of such thresholds. Predictive values (i.e. PPV) are influenced by disease prevalence, and an accurate test when used in a low prevalence population will have a poor PPV. In these populations, the NPV may be more accurate and clinically useful, especially if using this as a screening tool(47).

Secondly, when using an optimal cut-off in skin prick testing, there may be variability in results between extracts and between fresh tree nuts. The majority of cut-offs included in this systematic review use fresh tree nut, however there are different commercial extracts used. Ortolani's study is the only study using both fresh tree nut extract (hazelnut) and commercial extract(29). The commercial extracts performed similarly to fresh hazelnut from a diagnostic perspective with similar PPVs (0.93 vs 0.94 respectively) and sensitivity (0.90 vs 0.88), but negative predictive value using a 3mm cut-off was better using commercial extract (commercial extract 0.04, fresh hazelnut 0.15) as well as specificity (0.06 vs 0.28 respectively)(29). Commercial extracts for food are not standardized, and therefore without potency assessments -

standard measurement of the relevant antigens or the immune response(48). In other foods, Rance et al found a difference in labial food challenge with egg, peanut and cow' s milk. 52% of positive commercial extract SPT ($\geq 3\text{mm}$) correlated with a positive challenge, compared to 80% with fresh foods(49). Ruethers et al also showed poorer sensitivity with commercial extracts for fish. This study went further to analyze these extracts, demonstrating many commercial fish extracts contain insufficient amounts of allergens such as parvalbumin and collagen, resulting in low IgE reactivity and limited suitability for reliable in vivo testing(50). Other studies have also demonstrated poorer sensitivity with commercial extracts compared to the fresh food for cow' s milk, egg, wheat soy, peanut and kiwi, shrimp and wheat(51-55). On the other hand, concerns with using fresh foods revolve around safety, with case reports of anaphylaxis, and reliability and reproducibility of SPT using fresh foods(56). Such testing has not been performed in an appropriately designed study for tree nuts as yet, but fresh tree nuts have been suggested in preference to commercial extracts when performing SPT(57).

A third detail is the clinical use of BAT as a diagnostic tool. BAT remains essentially a research tool, with results suggesting similar sensitivity and specificity to other diagnostic tools in this analysis of tree nuts. One concern with BAT is the non-responder status, which can be seen in greater than 15% of patients in food allergy(58). In addition, the analysis needs to be performed on fresh blood samples. Just as OFC are resource intensive and put an individual at risk of severe reactions, BAT usefulness clinically will be limited by non-responders and the need for analysis to be done on fresh blood.

To have the greatest reflection of diagnostic accuracy of these tests, using a population-based cohort, or at minimum, a clinical outpatient cohort would be expected to provide the most reliable and clinically relevant results for the clinician. There are a number of studies included in this systematic review that retrospectively assess the outcome of clinician referred OFCs. Using this approach will give a selection bias to the patient population being assessed, and not be representative of the general population or those presenting to an allergy clinic for assessment. This may increase or decrease the accuracy of the test. Another issue is that the criteria to send someone for challenge, is often poorly described, therefore the sampling frame is unclear which reduces our ability to understand the generalizability of the study. In the retrospective analysis, the expectation is that most clinicians will err on the side

of 'at first do no harm' and refer patients for challenge that are most likely to pass. This may exclude patients with a high positive SPT or those with previous clinical reaction and ongoing low positive SPT results for example. The result is a higher specificity and lower sensitivity than what may be seen in a population cohort, or one where all positive SPT results went for OFC. Care should be taken when interpreting the above studies ('retrospective') and a comparison made where possible to a population-based cohort.

Using diagnostic algorithms such as those for the assessment of peanut allergy with Ara h 2(16) are likely where greatest diagnostic accuracy and reduced need for number of OFC are able to be achieved. For tree nuts, Elizur et al demonstrate a clinical algorithm for both assessment of walnut/pecan allergy and cashew/pistachio allergy. The combination of skin prick test and BAT correctly identified 87% of patients as allergic or tolerant to walnut, and 71% to cashew(14). Subsequently, a prospective study of the walnut/pecan algorithm report a 78.8% reduction in OFC, with 2.5% (6 of 240) false positive diagnoses and no false negative results(59). This algorithm, as well as others for other tree nuts, may significantly improve accuracy of allergy diagnosis with reduced dependence on OFC.

Strengths of this study include that it is a large and detailed systematic review based on the PRISMA guidelines, a widely recognized systematic review methodology. Whilst consensus of clear diagnostic cut-offs is not established for a number of tree nuts, it highlights the importance of interpreting any results with caution, and to consider the need for pre-emptive screening in this setting. It also highlights areas where future research may provide greater information and diagnostic power. For some foods which have a large volume of research (i.e. hazelnut) there may be clinical algorithms which will help to increase the predictive ability of these tests when used in combination.

Limitations of this study are that the studies have not been collated in a meta-analysis to assess an overall effect using pooled data. This was not undertaken as the studies reported different sensitivities and specificities at different thresholds. Predetermined thresholds such as $\geq 3\text{mm}$ are less effective as these may not be the optimal cut-off. Using only higher quality studies would have generally had only small or limited studies for each diagnostic test. Estimates of diagnostic accuracy of

certain tree nuts are limited by limited studies such as Almond, Pecan, Pistachio, Brazil nut and macadamia.

CONCLUSION

This systematic review has highlighted that although some of the tree nut diagnostic tests have 95% PPVs for determining clinical allergy, there is still a significant portion of individuals that will fall into the 'grey area', which ultimately requires an OFC to truly determine the individual's allergy status. There are clearly defined areas where testing has not been fully evaluated, which may help to reduce the number of OFCs required. Using diagnostic algorithms such as those demonstrated for walnut/pecan and cashew/pistachio allergy may achieve greater diagnostic accuracy and reduced number of OFCs. Ultimately through improved diagnostic accuracy with minimally invasive tests, there will be reduced need for OFCs and potentially a reduction in allergy rates and financial and health burdens of food allergy, which is an important consideration given increasing allergy prevalence worldwide.

726 **FIGURE LEGENDS (figures attached as separate files)**

727 Figure 1: Summary of search method

728

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Author	Risk of bias				Applicability concerns				Quality of study
	Patient Selection	Index test	Reference standard	Flow and timing	Patient Selection	Index test	Reference standard	Flow and timing	
McWilliam(15)	+	+	+	+	+	+	+	+	High
Elizur(14)	+	+	+	+	+	+	+	+	High
Cetinkaya(20)	+	+	+	+	+	+	+	+	High
Ho(21)	-	-	+	+	+	+	+	+	Low
Van Der Valk(60)	+	+	+	+	+	-	+	+	Low
Sato(22)	+	+	+	+	+	+	+	+	High
Savvatianos(23)	+	+	+	+	+	-	+	+	Low
Lange(24)	+	+	+	+	+	+	+	+	High
Blankestijn(25)	+	+	+	+	+	+	+	+	High
Elizur(13)	+	+	+	+	+	+	+	+	High
Sato(26)	+	+	+	+	+	+	+	+	High
Ortolani(29)	+	+	+	+	+	-	+	+	Low
Buyuktiryaki(27)	+	+	+	+	+	+	+	+	High
Eller(28)	-	-	+	+	+	+	+	+	Low
Beyer(30)	+	+	+	+	+	+	+	+	High
Carraro(31)	-	-	+	+	+	+	+	+	Low
Masthoff(32)	+	+	+	+	+	-	+	+	Low
Masthoff(33)	+	+	+	+	+	+	+	+	High
Brandstrom(34)	+	+	+	+	+	+	+	+	High
Inoue(61)	-	-	+	+	+	+	+	+	Low
Kattan(35)	-	-	+	+	+	-	+	+	Low
Virkud(37)	-	-	+	+	+	-	+	+	Low
Baker(36)	-	-	+	+	+	-	+	+	Low
Rayes(39)	+	+	+	+	+	-	+	+	Low

Table I: Reviewer's judgement about the risk of bias of studies included

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Skin Prick test							
								SPT Allergen	Reference cut-off	Method	Sens	Spec	PPV	NPV	AUC
McWilliam(15)*	2020	2011-2016	Retrospective and Population cohort	Australia	Open OFC or Clinical History	Mean (pop) 7.2; - Mean (clinic) 7.9; -	Cashew 167/531 (31.5%)	Cashew Extract -Clinician cohort: Hollister-Stier	Cashew Clin >14mm	95% PPV	0.08	0.99	0.95	0.80	0.81
								-Population cohort: ALK	Cashew Pop >10mm		0.43	0.98	0.95	0.48	0.89
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	Cashew 40/61 (65.6%)	Fresh Cashew	Cashew ≥8mm	ROC	0.83	0.91	-	-	0.94
									Cashew ≥12mm		-	-	0.95	-	
							Pistachio 26/65 (40%)	Fresh Pistachio	Pistachio ≥7mm	ROC	0.92	0.77			0.89
Cetinkaya(20)*	2019	2015-2017	Outpatient	Turkey	Open OFC	4.9; 3.4-7.4	Cashew 13/104 (12.5%)	Fresh Cashew	Cashew ≥6.25mm	Youden Index	0.92	0.79	0.6	0.97	0.90
							Pistachio 21/109 (19.2%)	Fresh Pistachio	Pistachio ≥7.25mm	Youden Index	0.82	0.75	0.55	0.92	0.85
Ho(21)	2006	1995-2005	Retrospective	Australia	Open OFC	-; 0.3-19	Cashew -/86 (-)	Extract Cashew: Dome-Hollister-Stier	Cashew ≥8mm	95% PPV	0.39	1.0	1.0	0.42	-
							Pistachio -/2 (-)	Extract Pistachio: Dome-Hollister-Stier	Pistachio ≥6mm	PPV	0.55	0.90	0.86	0.64	-
Van Der Valk(60)	2017	2012-2015	Outpatient	Netherlands	DBPCFC	9; 2-17	Cashew 137/179 (76.5%)	Fresh Cashew	≥3mm	ROC	0.99	0.33	0.86	0.93	-

Table IIa: Cashew and Pistachio Skin Prick Test (SPT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Whole nut IgE						
								Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV	AUC
Sato(22)*	2019	2010-2016	Outpatient	Japan	Open OFC	7; 2-26	Cashew 26/95 (27.4%)	Cashew >2.62	ROC	0.81	0.75	-	-	0.79
Cetinkaya(20)*	2019	2015-2017	Outpatient	Turkey	Open OFC	4.9; 3.4-7.4	Cashew 13/104 (12.5%)	Cashew ≥1.125	Youden Index	1.0	0.54	0.41	1.0	0.79
							Pistachio 21/109 (19.3%)	Pistachio ≥4.14	Youden Index	0.77	0.53	0.38	0.87	0.67
Savvatanos(23)	2015	2010-2012	Outpatient	Greece	Open OFC or Clinical History	Allergic 6.5; 4.1-9.1 Tolerant 9.8; 5.8-13.3	Combined 100/136 (73.5%) Cashew allergic only 38 Pistachio allergic only 37 Allergic to both 25	Cashew ≥0.35	ROC	0.95	0.58	-	-	0.89
								Pistachio ≥0.35	ROC	0.97	0.44	-	-	0.88

Table IIb: Cashew and Pistachio IgE (whole nut)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Nut component IgE						
								Type	Reference cut-off (kUA/L)	Method	Sens	Spec	PPV	AUC
Lange(24)*	2017	-	Outpatient	Germany	DBPCFC or Clinical History	Allergic 3.8; IQR 2.2-6.3 Tolerant 6.7; IQR 3.5-13.2	Cashew 42/61 (68.9%)	Ana o 3	Cashew ≥ 0.3	ROC	0.93	0.90	-	0.94
									Cashew ≥ 2.0		-	-	0.95	
Sato(22)*	2019	2010-2016	Outpatient	Japan	Open OFC	7; 2-26	Cashew 26/95 (27.4%)	Ana o 3	Cashew ≥ 0.35	ROC	0.85	0.77	-	0.92
									Cashew $\geq 0.70^*$		0.85	0.88	-	
Savvatanos(23)	2015	2010-2012	Outpatient	Greece	Open OFC or Clinical History	Allergic 6.5; 4.1-9.1 Tolerant 9.8; 5.8-13.3	Combined 100/136 (73.5%) Cashew allergic only 38 Pistachio allergic only 37 Allergic to both 25	Ana o 3	Cashew ≥ 0.35	ROC	0.94	0.94	-	0.98
									Cashew $\geq 0.16^{**}$		0.98	0.94	-	
									Pistachio ≥ 0.35	ROC	0.92	0.94	-	0.97

									Pistachio $\geq 0.16^{**}$	0.97	0.94	-	
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Table IIc: Cashew and Pistachio Component IgE (CRD)

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Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Basophil Activation Test					
								Reference cut-off	Method	Sensitivity	Specificity	PPV	AUC
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	Cashew 40/61 (65.6%)	Cashew $\geq 2\%$ CD63+	ROC	0.77	0.93	-	0.97
								Cashew $\geq 5.5\%$ CD63+		-	-	0.95	
							Pistachio 26/65 (40%)	Pistachio $\geq 5.8\%$ CD63+	ROC	0.77	0.88	-	0.80

Table IId: Cashew and Pistachio Basophil Activation Testing (BAT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Skin Prick test							
								SPT Allergen	Reference cut-off	Method	Sens	Spec	PPV	NPV	AUC
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	Walnut 53/71 (74.6%)	Fresh Walnut	Walnut ≥6mm	ROC	0.87	0.79	-	-	0.89
								Walnut ≥8mm	-		-	0.95	-		
							Pecan 34/57 (59.6%)	Fresh Pecan	Pecan ≥5mm	ROC	0.83	0.79	-	-	0.84
Blankestijn(25)*	2017	2012-2015	Outpatient	Netherlands	DBPCFC	32; IQR 25-38	Walnut 33/55 (60%)	Walnut Extract (ALK1)	Walnut ≥5mm	Youden Index	0.55	0.95	0.58	0.58	0.80
									Walnut ≥8mm		0.27	1.0	1.0	0.48	
Ho(21)	2006	1995-2005	Retrospective	Australia	Open OFC	-; 0.3-19	Walnut -/29 (-)	Walnut Extract (Dome-Hollister-Stier)	Walnut ≥8mm	95% PPV	0.14	1.0	1	0.54	-
							Pecan -/14 (-)	Pecan Extract (Dome-Hollister-Stier)	Pecan ≥7mm	PPV	0.5	0.96	0.75	0.90	-

Table IIIa: Walnut and Pecan Skin Prick Test (SPT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Whole nut IgE						
								Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV	AUC
Blankestijn(25)*	2017	2012-2015	Outpatient	Netherlands	DBPCFC	32; IQR 25-38	Walnut 33/55 (60%)	Walnut ≥ 0.46	Youden Index	0.67	0.91	0.92	0.65	0.86
Elizur(13)*	2020	2016-2018	Outpatient	Israel	Open OFC or Clinical History	8; 4-24	Walnut 61/76 (80.3%)	Walnut ≥ 5.07	ROC	0.62	0.87	0.95	0.36	0.84
Sato(26)*	2017	-	Outpatient	Japan	DBPCFC or Open OFC	(allergic 6, non-allergic 7); 2-20	Walnut 60/108 (55.6%)	Walnut > 2.58	ROC	0.73	0.70	0.75	0.67	0.79

Table IIIb: Walnut and Pecan IgE (Whole Nut)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Nut component IgE							
								Type	Method	Reference cut-off (kUA/L	Sensitivity	Specificity	PPV	NPV	AUC
Blankestijn(25)*	2017	2012-2015	Outpatient	Netherlands	DBPCFC	32; IQR 25-38	Walnut 33/55 (60%)	Jug r 1	Youden Index	≥0.1	0.61	0.91	0.91	0.61	0.80
Elizur(13)*	2020	2016-2018	Outpatient	Israel	Open OFC or Clinical History	8; 4-24	Walnut 61/76 (80.3%)	Jug r 1	ROC	≥0.21	0.87	0.8	0.95	0.6	0.85
								Jug r 4		≥3	0.31	0.93	0.95	0.25	0.73
Sato(26)*	2017		Outpatient	Japan	DBPCFC or Open OFC	(Allergic 6, non-allergic 7); 2-20	Walnut 60/108 (55.6%)	Jug r 1	ROC	≥0.423	0.85	0.79	0.84	0.81	0.84

Table IIIc: Walnut and Pecan Component IgE (CRD)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n (% allergic)	Basophil Activation Test					
								Reference cut-off	Method	Sensitivity	Specificity	PPV	AUC
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	Walnut 53/71 (74.6%)	Walnut $\geq 6.4\%$ CD63+	ROC	0.8	0.88	0.95	0.86
							Pecan 34/57 (59.6%)	Pecan $\geq 2.7\%$ CD63+		0.74	0.83	-	0.78

Table IIIId: Walnut and Pecan Basophil Activation Testing (BAT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Skin Prick test							
								SPT Allergen	Reference cut-off	Method	Sensitivity	Specificity	PPV	NPV	AUC
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	14/61 (23%)	Fresh Hazelnut	$\geq 8\text{mm}$	ROC	0.64	0.77	0.38	0.91	0.75
Buyuktiyaki(27)*	2016	2011-2014	Outpatient	Turkey	DBPCFC or Clinical History	3.4; 2.1-7.2	32/64 (50%)	Hazelnut Extract (Alyostal)	$\geq 7.5\text{mm}$	Youden Index	0.588	0.90	0.91	0.56	0.80
Ortolani(29)	2000	-	Outpatient	Switzerland, Germany, Denmark	DBPCFC	30; 14-64	67/86 (77.9%)	Hazelnut Extract (Multiple)	$\geq 3\text{mm}$	Unclear if optimal	0.90	0.06	0.93	0.04	-
								Fresh Hazelnut	$\geq 3\text{mm}$		0.88	0.28	0.94	0.15	-
Eller(28)	2016	2001-2010	Retrospective	Denmark	DBPCFC or Open OFC	5.1; 0.7-15.5	65/155 (41.9%)	Fresh Hazelnut	$\geq 7\text{mm}$	ROC	0.62	0.81	-	-	0.79
Ho(21)	2006	1995-2005	Retrospective	Australia	Open OFC	-; 0.3-19	-/58 (-)	Hazelnut Extract (Dome-Hollister-Stier)	$\geq 8\text{mm}$	95% PPV	0.08	1.00	1.00	0.79	-

Table IVa: Hazelnut Skin Prick Test (SPT)

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Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Whole nut IgE						
								Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV	AUC
Buyuktiryaki(27)*	2016	2011-2014	Outpatient	Turkey	DBPCFC or Clinical History	3.4; 2.1-7.2	32/64 (50%)	≥3.15	Youden Index	0.71	0.91	0.85	0.81	0.82
Beyer(30)*	2015		Outpatient	Germany	DBPCFC or Open OFC	4.5;2.1-8.1	44/143 (30.8%)	≥0.35	ROC	0.98	0.21	0.36	0.97	0.71
Masthoff(32)	2012	2004-2010	Outpatient	Netherlands	DBPCFC	6; IQR 5-9	51/151 (33.8%)	≤0.35	ROC	-	-	-	0.95	-
Carraro(31)	2016	2013-2014	Retrospective	Italy	Open OFC	-; 3-14	24/36 (66.7%)	≥7.25	ROC	0.79	0.83	-	-	0.87
Ortolani(29)	2000	-	Outpatient	Switzerland, Germany, Denmark	DBPCFC	30; 14-64	67/86 (77.9%)	≥0.7	Unclear if optimal	0.75	0.17	0.92	0.05	-

Table IVb: Hazelnut IgE (Whole Nut)

Journal Pre-proof

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Nut component IgE							
								Type	Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV	AUC
Masthoff(33)*	2013	2010-2012	Outpatient	Netherlands	DBPCFC	8; IQR 7-12	40/81 (49.4%)	Cor a 9	≥1.0	ROC	0.75	0.95	-	-	0.87
								Cor a 14	≥5.0		0.60	0.98	-	-	0.80
Brandstrom(34)*	2015	-	Outpatient	Sweden	DBPCFC	11; 6-18	8/40 (20%)	Cor a 9	>0.65	ROC	1.0	0.72	0.47	1.0	0.87
								Cor a 14	>0.64		1.0	0.94	0.8	1.0	0.98
Beyer(30)*	2015	-	Outpatient	Germany	DBPCFC or Open OFC	4.5; 2.1-8.1	44/143 (30.8%)	Cor a 14	≥0.35	ROC	0.85	0.81	0.64	0.91	0.89
Buyuktiryaki(27)*	2016	2011-2014	Outpatient	Turkey	DBPCFC or Clinical History	3.4; 2.1-7.2	32/64 (50%)	Cor a 14	≥0.63	Youden Index	0.82	1.0	1.0	0.86	0.94
Eller(28)	2016	2001-2010	Retrospective	Denmark	DBPCFC or Open OFC	5.1; 0.7-15.5	65/155 (41.9%)	Cor a 9	≥1.37	ROC	0.63	0.83	-	-	0.78
								Cor a 14	≥0.72		0.80	0.92	0.92	-	0.85
Carraro(31)	2016	2013-2014	Retrospective	Italy	Open OFC	-; 3-14	24/36 (66.7%)	Cor a 9	>1.01	ROC	0.75	0.75	-	-	0.88
								Cor a 14	>1.93		0.96	0.92	-	-	0.98
Inoue(61)	2020	2006-2017	Retrospective	Japan	Open OFC	7.3; IQR 5.9-10.5	9/91 (9.9%)	Cor a 1	<4.67	ROC	-	-	-	0.95	0.72
								Cor a 9	<0.23		-	-	-	0.95	0.71
Kattan(35)	2014	2010-2013	Retrospective	United States	Open OFC	9; -	9/116 (7.8%)	Cor a 9	≥2.0	-	0.85	0.93	-	-	-
								Cor a 14	≥1.0		0.69	1.0	-	-	-

Table IVc: Hazelnut Component IgE (CRD)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Basophil Activation test				
								Reference cut-off	Method	Sensitivity	Specificity	AUC
Elizur(14)*	2018	2016-2017	Outpatient	Israel	Open OFC or Clinical History	8.7; 3-24	14/61 (23%)	≥3.4% CD63+	ROC	1.0	0.87	0.94
Brandstrom(34)*	2015	-	Outpatient	Sweden	DBPCFC	11; 6-18	8/40 (20%)	>1.7% CD63+	ROC	1.0	0.97	0.98

Table IVd: Hazelnut Basophil Activation Testing (BAT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Skin Prick test								
								SPT Allergen	Challenge Outcomes*	Reference cut-off	Method	Sensitivity	Specificity	PPV	NPV	AUC
Ho(21)	2016	1995-2005	Retrospective	Australia	Open OFC	-; 0.3-19	-/28 (-)	Almond Extract (Dome-Hollister-Stier)	-	≥7mm	PPV	0.33	0.92	0.33	0.92	-
Virkud(37)	2019	2009-2018	Retrospective	United States	Open OFC	10.2 (mean); -	30/590 (5.1%)	Not disclosed	OFC pass rate depending on SPT size ≤2mm: 97% 3-4mm: 97% 5-6mm: 93% 7-8mm: 90% >8mm: 90%	11mm	-	0.14	0.99	0.36	0.95	0.66
										46mm		-	-	0.95	-	
Baker(36)	2019	2015-2017	Retrospective	United States	Open OFC	7.35; 0.6-25	16/400 (4%)	Not disclosed	OFC pass rate depending on SPT size ≤5mm: 97.2% 6-8mm: 92.6% ≥9mm: 81.8%	-	-	-	-	-	-	-

Table Va: Almond Skin Prick Test (SPT)

Journal Pre-proof

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Whole nut IgE							
								Challenge Outcomes*	Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV	AUC
Virkud(37)	2019	2009-2018	Retrospective	United States	Open OFC	10.2 (mean); -	30/590 (5.1%)	OFC pass rate depending on sIgE level (kUA/L) <0.35: 97% 0.35-1.00: 97% 1.01-2.00: 88% 2.01-10.00: 97% >10: 83%	≥10	-	0.21	0.93	0.15	0.95	0.61
									≥174		-	-	0.95	-	
Baker(36)	2019	2015-2017	Retrospective	United States	Open OFC	7.35; 0.6-25	16/400 (4%)	OFC pass rate depending on sIgE level (kUA/L) <0.35: 100% 0.35-1.99: 97% 2.0-4.99: 95.8% 5-10.0: 96.8% >10: 86.4%	-	-	-	-	-	-	-

Table Vb: Almond IgE (Whole Nut)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Skin Prick test						
								SPT Allergen	Reference cut-off	Method	Sensitivity	Specificity	PPV	NPV
Ho(21)	2006	1995-2005	Retrospective	Australia	Open OFC	-; 0.3-19	-/10 (-)	Brazil nut extract (Dome-Hollister-Stier)	≥8mm	PPV	0.75	0.95	0.75	0.95

Table VIa: Brazil Nut Skin Prick Test (SPT)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Whole nut IgE						
								Reference cut-off	Method	Sensitivity	Specificity	PPV	NPV	

Rayes(39)	2016	1998-2000	Outpatient	United Kingdom	DBPCFC or Clinical History	18; IQR 10-35	19/34 (55.9%)	≥1.51kUA/L	ROC	0.42	0.93	0.89	0.56
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Table VIb: Brazil Nut IgE (Whole Nut)

Author	Published year	Data collection years	Population	Country	Reference standard	Age, years (median; span)	Participants, allergic/total n(% allergic)	Nut component IgE						
								Type	Reference cut-off (kUA/L)	Method	Sensitivity	Specificity	PPV	NPV
Rayes(39)	2016	1998-2000	Outpatient	United Kingdom	DBPCFC or Clinical History	18; IQR 10-35	20/36 (55.6%)	Baseline	0.25kUA/L	ROC	0.75	0.94	0.94	0.75

Table VIc: Brazil Nut Component IgE (CRD).

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Legends

Table 1: Reviewer's judgement about the risk of bias of studies included. Supplemental table E2 gives a detailed explanation of domains for risk of bias, using the QUADAS-2 tool(19). '+' , low risk; '-' , high risk.

Table IIa: Cashew and Pistachio Skin Prick Test (SPT). *Method*, Method calculating optimal cut-off; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; '*' , indicating a study of high quality, based on quality assessment (Table 1); *Cashew Clin*, Retrospective cohort with cashew allergy; *Cashew Pop*, Population-based cohort with cohort allergy. Skin prick test extracts: *Hollister-Stier*, Hollister-Stier, Stallergenes, Antony, France; *ALK*, ALK-Abello SA, Madrid, Spain; *Dome-Hollister-Stier*, Dome-Hollister-Stier, Spokane, Washington, United States

Table IIb: Cashew and Pistachio IgE (whole nut). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; '*' , indicates a study of high quality, based on quality assessment (Table 1).

Table IIc: Cashew and Pistachio Component IgE (CRD). *Method*, Method calculating optimal cut-off; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive Predictive Value; *AUC*, Area under the curve; '*' , indicates a study of high quality, based on quality assessment (Table 1); "***" indicates the optimal cut-off.

Table IId: Cashew and Pistachio Basophil Activation Testing (BAT). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *AUC*, Area under the curve; '*' , indicates a study of high quality, based on quality assessment (Table 1); *CD63*, CD63+ expression on Basophils.

Table IIIa: Walnut and Pecan Skin Prick Test (SPT). *Method*, Method calculating optimal cut-off; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; '*' , indicates a study of high quality, based on quality assessment (Table 1). Skin prick test extracts: *ALK1*, ALK-Abello, Hørsholm, Denmark; *ALK2*, ALK-Abello, Madrid, Spain; *Dome-Hollister-Stier*, Dome-Hollister-Stier, Spokane, Washington, United States.

Table IIIb: Walnut and Pecan IgE (Whole Nut). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; '*' , indicates a study of high quality, based on quality assessment (Table 1).

Table IIIc: Walnut and Pecan Component IgE (CRD). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1).

Table IIIId: Walnut and Pecan Basophil Activation Testing (BAT). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1); *CD63+*, CD63+ expression on Basophils.

Table IVa: Hazelnut Skin Prick Test (SPT). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1). Skin prick test extracts: *Alyostal*, Alyostal ST-IR, Stallergenes SA, Antony, France; *Multiple*, Multiple extracts used, with pooled data. Extracts included: Lofarma Spa, Milan, Italy, Stallergenes, Antony Cedex, France, ALK-Abello, Hordholm, Denmark; *Dome-Hollister-Stier*, Dome-Hollister-Stier, Spokane, Washington, United States.

Table IVb: Hazelnut IgE (Whole Nut). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1).

Table IVc: Hazelnut Component IgE (CRD). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1).

Table IVd: Hazelnut Basophil Activation Testing (BAT). *Method*, Method calculating optimal cut-off; *AUC*, Area under the curve; ‘*’, indicates a study of high quality, based on quality assessment (Table 1); *CD63+*, CD63+ expression on Basophils.

Table Va: Almond Skin Prick Test (SPT). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve; Skin prick test extracts: *Dome-Hollister-Stier*, Dome-Hollister-Stier, Spokane, Washington, United States.

Table Vb: Almond IgE (Whole Nut). *Method*, Method calculating optimal cut-off; *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; *AUC*, Area under the curve.

244 Table VIa: Brazil Nut Skin Prick Test (SPT). *Method*, Method calculating optimal cut-off;
245 *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value; Skin Prick test extracts:
246 *Dome-Hollister-Stier*, Dome-Hollister-Stier, Spokane, Washington, United States.

247

248 Table VIb: Brazil Nut IgE (Whole Nut). *Method*, Method calculating optimal cut-off; *PPV*,
249 Positive Predictive Value; *NPV*, Negative Predictive Value.

250

251 Table VIc: Brazil Nut Component IgE (CRD). *Method*, Method calculating optimal cut-off;
252 *PPV*, Positive Predictive Value; *NPV*, Negative Predictive Value.

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1. Nut Hypersensitivity/
2. (nut or nuts or cashew* or pistachio* or walnut* or pecan* or hazelnut* or hazel-nut* or almond* or brazilnut* or brazil-nut* or macadamia*).tw,kf.
3. (seed-storage-protein* or 2S-albumin* or vicilin* or legumin*).tw,kf.
4. (ana-o* or pis-v* or jug-r-1 or jug-r1 or jug-r-2 or jug-r2 or jug-r-3 or jug-r3 or jug-r-4 or jug-r4 or car-i-1 or car-i1 or car-i-4 or car-i4 or cor-a-1 or cor-a1 or cor-a-9 or cor-a9 or cor-a-14 or cor-a14 or pru-du*).tw,kf.
5. (food-challenge* or skin-prick* or skin-test* or IgE* or component-resolved* or basophil-activation*).tw,kf.
6. (ALLERG* or HYPERSENSITIV* or HYPER-SENSITIV* or sensitisation* or sensitization* or anaphyla*).tw,kf,hw.
7. "sensitivity and specificity"/ or "predictive value of tests"/ or roc curve/
8. exp cohort studies/
9. (longterm or long-term or repeat* or serial or longitudinal* or follow-up or followup or cohort or retrospect* or prospect* or probabilit* or sensitivit* or specificit* or predictive).tw,kf.
10. basophil degranulation test/ or serologic tests/ or exp skin tests/
11. Immunoglobulin E/
12. anacardium/ae or pistacia/ae or exp Juglandaceae/ae or Corylus/ae or prunus dulcis/ae or bertholletia/ae
13. (OFC or BAT or molecular-based or utilit*).tw,kf.
14. Nuts/
15. (2 or 3 or 4 or 14) and 6
16. 1 or 12 or 15
17. 5 or 10 or 11 or 13
18. 7 or 8 or 9
19. 16 and 17 and 18
20. di.fs.
21. (sensitivity or specificity).ti,ab.
22. likelihood functions/ or probability/
23. (likelihood adj ratio*).ti,ab.
24. diagnos*.tw,kf.
25. 16 and 17 and (8 or 9) and (7 or 20 or 21 or 22 or 23 or 24)

Figure E1

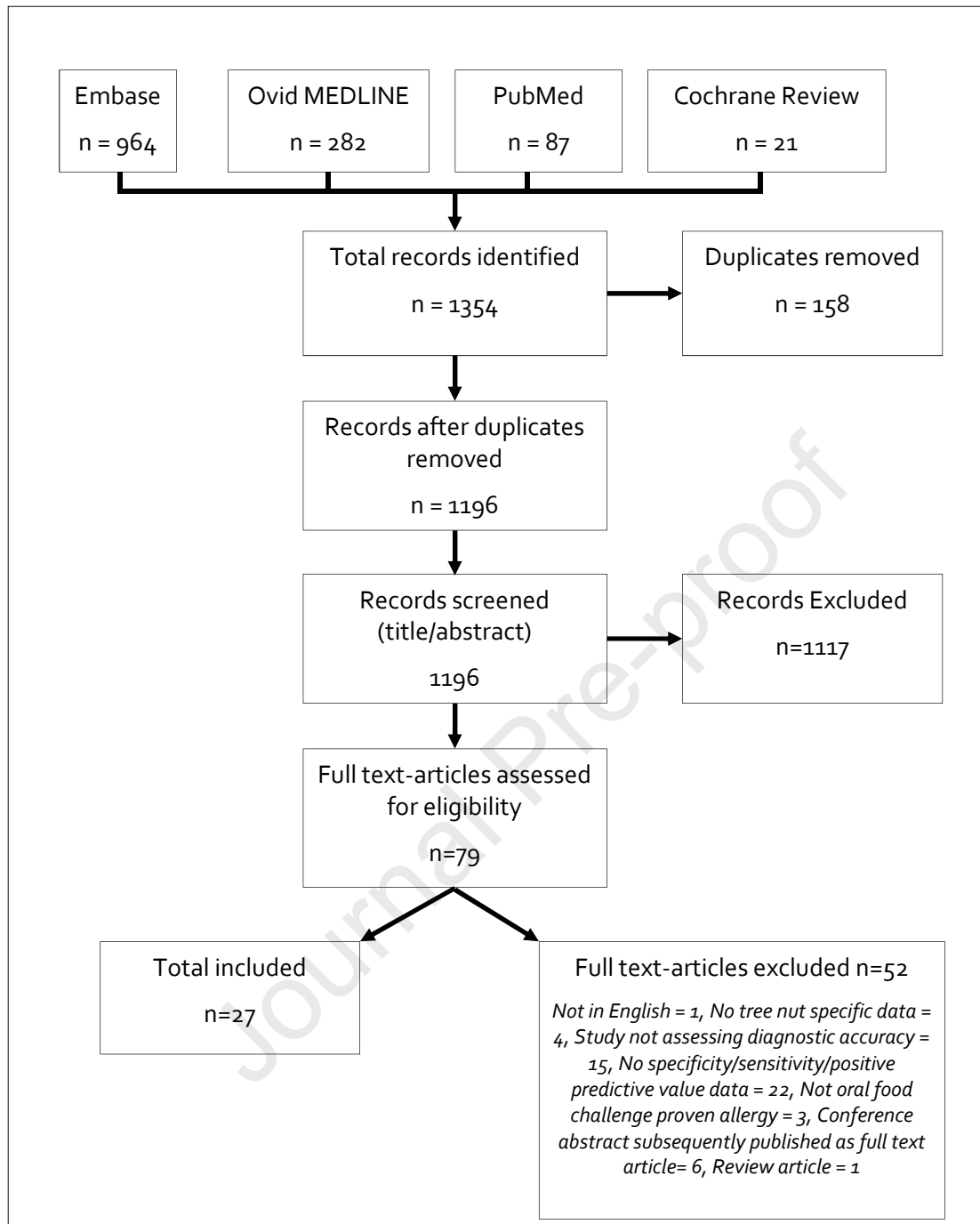


Figure 1: Summary of search method