

Review

Diagnosis of Food Allergy: Which Tests Truly Have Clinical Value?

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Abstract

Food allergy diagnosis remains challenging due to the difficulty of distinguishing true clinical allergy from asymptomatic sensitization. Inaccurate diagnosis may result in unnecessary dietary restrictions, reduced quality of life, or, conversely, failure to identify individuals at risk of severe allergic reactions. This review critically analyzes the efficacy, limitations, and clinical utility of currently available diagnostic tests for food allergy, with particular emphasis on their ability to predict true clinical reactivity. A comprehensive literature review was conducted to evaluate the sensitivity, specificity, and predictive values of both traditional and emerging diagnostic modalities. English-language guidelines, systematic reviews, and key clinical studies published primarily within the past 15 years (up to 2025) were identified through PubMed and Google Scholar. Classic diagnostic tools, including skin prick testing (SPT) and serum-specific IgE (sIgE), were assessed alongside novel approaches such as component-resolved diagnostics (CRD), basophil activation test (BAT), mast cell activation test (MAT), atopy patch testing (APT), cytokine profiling, and omics-based diagnostics. Particular attention was given to how these tests compare with the oral food challenge (OFC), which remains the diagnostic gold standard. The findings demonstrate that while conventional tests offer high sensitivity and are valuable for initial risk assessment, their limited specificity often leads to overdiagnosis. Emerging molecular and cellular assays show improved specificity and functional relevance, especially in complex cases involving polysensitization or unclear clinical histories and may reduce reliance on OFCs in the future. However, accessibility, cost, and lack of standardization currently limit their widespread clinical application. Advances in artificial intelligence and data integration hold promise for improving diagnostic accuracy through enhanced interpretation of complex immunological data. Based on the synthesized evidence, this review proposes an evidence-based, stepwise, and individualized diagnostic algorithm for food allergy. Integrating clinical history, targeted testing, and selective use of OFCs can improve diagnostic certainty, enhance food safety, minimize unnecessary dietary avoidance, and optimize patient outcomes. The review underscores the need for continued research,



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standardization, and validation of novel diagnostic tools to support personalized and precise food allergy management.

Keywords: food allergy; diagnosis; diagnostic test; immunological markers; diagnostic algorithm

1. Introduction

Food allergies have become an escalating health concern with increasing prevalence over the past decades, causing significant impairments in physical and psychological health in individuals. This burden often extends to affected individuals, their families, caregivers, and the healthcare system as a whole [1–3]. Accurate diagnosis of food allergy is critical to patient care, as it can prevent both life-threatening allergic reactions and unnecessary dietary restrictions that negatively impact quality of life [4].

Despite advances in the field, food allergy diagnosis remains challenging. Both underdiagnosis and overdiagnosis are common largely due to limitations in currently available diagnostic tools. A central priority in contemporary allergology is identifying which tests provide meaningful clinical information, discriminate well between sensitization and clinical allergy, and accurately predict the severity of the reaction is one of the main priorities in current allergology [1–3].

Food allergy diagnostics require a multifaceted approach that combines a comprehensive clinical history, physical examination, and the use of *in vivo* and *in vitro* tests. Clinical history alone often lacks sufficient sensitivity and interpretation [5]. A key consideration is differentiating between IgE-mediated and non-IgE-mediated food allergy, as the pathophysiology differs and subsequent hypersensitivity reactions can vary significantly [6–8].

In suspected IgE-mediated food allergies, the presence of allergen-specific IgE antibodies (sensitization) is usually assessed through skin prick tests (SPTs) or serum specific IgE (sIgE) assays [5]. However, sensitization does not necessarily equate to clinical allergy: patients may be sensitized (test positive) without being clinically allergic, and conversely, rare cases of true allergy can occur without detectable IgE.

The Oral Food Challenge (OFC) remains the diagnostic gold standard for confirm or exclude the presence of a food allergy [6–8]. Nevertheless, food challenges are time-consuming and carry the risk of severe reactions, so clinicians rely on less risky tests to guide the decision for a challenge. It is therefore important to carefully assess the available diagnostics for food allergy—Skin Prick Test (SPT), serum specific Immunoglobulin E (sIgE), Component-Resolved Diagnostics (CRD), Basophil Activation Test (BAT), etc.—to identify the test with the most reliable information [6–8].

Not all tests are equally useful—some provide strong predictive value for clinical allergy, while others have limited or no proven utility in routine practice. This review summarizes the clinical value and accuracy of various diagnostic methods for food allergy, highlighting which tests are truly beneficial and how they should be used [3,6,7].

2. Pathophysiological Background of Food Allergy

The immunological mechanisms responsible for food allergies are complex and involve different immunological responses to food-derived proteins (allergic triggers). The most rapid type of food-allergic reaction is characterized by IgE-mediated responses to food antigens, which, in most cases, are life-threatening. After the IgE antibody sensitization phase, the binding of food antigen-specific IgE antibodies to the high-affinity receptor FcεRI on mast cells and basophils (effector cells) leads to degranulation with the release of vasoactive mediators such as histamine, prostaglandins, and leukotriene. Subsequently,

the release of these vasoactive mediators leads to rapid symptoms, including urticaria, angioedema, bronchospasm, and anaphylaxis, requiring accurate diagnosis of the causal food triggers [7]. Since the reactions have a rapid onset and systemic involvement, accurate, rapid diagnosis is crucial for patient safety and quality of life [5,8].

Most of the symptoms of the IgE-mediated food allergy reaction are due to the properties of mast cells and basophils. The distribution of these cells amongst different tissues along with mediator release explains the diverse range of clinical symptoms with rapid-onset reactions that occur in food allergy. Given their central role in the disease pathogenesis, mast cells and basophils play an important part in functional tests, like the basophil activation test (BAT), to determine the activation of basophils in response to a specific food allergen, confirming IgE-mediated hypersensitivity and allowing a diagnostic role, in addition to skin prick test (SPT) and serum specific IgE (sIgE) assays [9]. Additional work on its clinical validity is warranted due to limitations, such as the requirement of fresh blood samples, specialized laboratories, and costs of implementation [7,10].

Food-allergic reactions that are mediated by mechanisms other than IgE, with slow and delayed symptom onset, typically include food protein-induced enterocolitis syndrome (FPIES), allergic proctocolitis, and eczema, a flare of atopic dermatitis induced by a food allergen. These reactions do not show any food antigen-specific IgE or mast cell degranulation and therefore have limited diagnostic usefulness with SPT and sIgE measurements [11]. Given the delayed symptom onset, with isolated tissue inflammation, other reliable tests are lacking, and most diagnoses rely on elimination diets and oral food challenges, which are associated with substantial risks and burdens [7,12].

The immunological basis for non-IgE-mediated responses follows the classical model of antigen presentation to T lymphocytes, leading to their activation and cytokine production, which induces the recruitment of other effector cells. The response leads to inflammation confined to particular tissues, and the delayed symptom onset is thought to be driven by the insufficient performance of diagnostic tests that predominantly focus on IgE-mediated mechanisms [11]. Therefore, there is a dire need for non-IgE-specific biomarkers and diagnostic tests that can enhance diagnostic capability. The poor diagnostic utility often delays the diagnosis of these allergies, potentially causing more morbidity and unnecessarily long-term diet restrictions [3,7].

In non-IgE-mediated gastrointestinal food allergies, symptom onset is typically delayed (hours to days), and diagnosis remains largely clinical—based on symptom improvement after elimination of the culprit food and recurrence upon reintroduction [13,14].

For food protein-induced enterocolitis syndrome (FPIES), recent syntheses highlight variability in triggers and natural history, reinforcing the importance of standardized diagnostic criteria and age-appropriate oral food challenge protocols [15,16].

The differentiation between IgE- and non-IgE-mediated reactions to foods is crucial, since IgE-specific diagnostic tools are ineffective in non-IgE-mediated reactions. This nuance can lead to wrong diagnoses (false negatives) if these tools are used in non-IgE-mediated food allergy reactions [7,17,18].

Accurate diagnosis of food allergy requires a pathway of testing and interpretation for each presentation of food allergy. The advent of molecular diagnostics, specifically component-resolved diagnostics (CRD), has allowed the differentiation between patients with true or primary allergy to a food compared to patients with only pollen or cross-reactive carbohydrate determinant (CCD) cross-reactive antibodies, thus leading to a more precise diagnosis and fewer unnecessary restrictions [7,17,18].

Molecular diagnostics are able to precisely identify the sensitization profile, where it has been found that most allergic patients are poly-sensitized but not necessarily poly-allergic to foods and pollen. In peanut allergy, for example, IgE to Ara h 2, a peanut storage

protein, indicates a severe peanut allergy. In contrast, high IgE levels to the CCD, contained within many allergenic plant extracts, usually indicate mild to no symptoms after peanut ingestion [17]. By targeting individual allergen protein components rather than to crude allergen extracts, these tools enable more precise analysis, and the specificity and resolution of testing have improved considerably. The impact on the prediction of reaction severity is promising, enabling better and safer medical intervention for patients, decreasing dietary restrictions, and improving the overall quality of life in affected individuals [7,18].

Molecular diagnostics have allowed us to better understand the phenomenon of cross-reactivity, where the IgE antibody cross-binds to more than one antigen that has a similar protein structure from different allergenic sources, often from pollen allergens and related plant-derived food sources. IgE specific to peach lipid transfer protein (Pru p 7) cross-reacts with certain pollen, which in some instances increases the risk, but also raises issues in interpretation and diagnostic confusion. The diagnostic ability that CRD brings into allergy practice is clear, as allergy practitioners are in a much better position to advise on the prevention of reactions to other pollen-related food plants [17]. In addition, the improved diagnostic power of CRD goes hand-in-hand with the general goals of precision medicine as the tests allow health professionals to offer personalized counseling and advice that address their patients' concerns by identifying allergens of real clinical significance [7,18].

Allergic diseases are heterogeneous; that is, many different pathways are involved, and the relative involvement of any individual path in the overall presentation varies between individuals. Clinical symptoms of food allergies are affected by the magnitude and quality of the immune response to foods. The extent to which the inflammatory cascade affects a particular tissue or organ system may vary greatly from one person to another, influencing the severity of symptoms, the time of onset, and whether patients outgrow a food allergy or not [9]. Similarly, different individuals elicit predominantly Th2 cytokine, or more of a mix of Th2 and Th1 cytokines (interleukin 4, interleukin 5, interleukin 13 and interferon γ , interleukin 2, tumor necrosis factor β), upon exposure to a food allergen, which further illustrates the wide spectrum of reactions and the differences among individuals with allergic disease. There is also a different spectrum of immune cells with each phenotype [3,19].

This suggests that the typical "one-size-fits-all" approach to diagnostics may not be effective, or it may be a case that the majority will only need certain aspects of each step of the pathway to accurately determine a clinical diagnosis. However, not every patient will be similar enough to make an accurate diagnosis without personalized protocols for each of them. Some evidence from other conditions supports the role of integrating novel tools into diagnostic pathways in the form of cytokine expression profiles and effector cell function, allowing more precise identification of atypical phenotypes, enhanced predictions of symptom severity, and support for the development of treatment plans [9]. The field is advancing towards the goal of developing strategies for the personalized diagnosis and treatment of each patient by integrating immune profiles and in vitro basophil function, leading to identification of clinical subgroups. For instance, heterogeneity in effector cell responsiveness or in the production of specific cytokines after food exposure may serve as thresholds for diagnostic testing [7,10].

Moreover, a subset of patients shows mixed patterns of responses (i.e., both IgE- and cell-mediated reactions to foods, with variable clinical manifestations that may change over time). The classification system for allergic disease has also proven insufficient to address mixed forms, and there is a need to incorporate clinical, immunological, and molecular components to the diagnostic classification for effective approaches to treatment [9,11]. Overall, there is evidence of increased complexity with the presentation and diagnosis of food allergies than previously thought [7].

Several aspects of the diagnosis of food allergy remain an unsolved riddle, as many challenges hinder diagnostic validity, like the incomplete understanding of the interaction between the innate and adaptive immune system in food allergy or how the human microbiome can influence food allergy. Moreover, current protocols for SPT, oral food challenges, and serum sIgE often yield ambiguous results [7,12,20].

As a result, misdiagnoses occur and ineffective therapies are employed, further burdening patients and healthcare providers. Novel technologies and transdisciplinary research are needed to explore new biomarkers for diagnostics and monitor allergic disease outcomes [7,12,20].

Updated clinical guidelines that incorporate new clinical data and new technologies should be issued periodically to promote uniformity in the assessment of allergic disease across practices [7,12,20].

The classification of food allergies is presented in Table 1.

Table 1. Classification of Food Allergies [3,7,12,17,18,20].

Allergy Type	Immunological Mechanism	Typical Onset	Symptoms	Clinical Examples
IgE-mediated	Immediate hypersensitivity via allergen-specific IgE bound to mast cells/basophils; cross-linking triggers mediator release	Seconds to minutes after exposure	Acute urticaria, angioedema, itching; respiratory (wheeze, cough); gastrointestinal (vomiting, diarrhea, pain); anaphylaxis possible	Peanut, tree nut, shellfish, fish, milk, egg and other common food allergens (the “top 9” foods)
Non-IgE-mediated	Delayed hypersensitivity primarily mediated by T cells and eosinophils; no specific IgE involvement	Hours to days after ingestion	Predominantly gastrointestinal: vomiting, diarrhea, abdominal pain; may include skin (eczema) or respiratory symptoms; anaphylaxis is rare	Food protein-induced enterocolitis (FPIES), proctocolitis (FPIAP), enteropathy (FPE); celiac disease; other non-IgE gastrointestinal disorders
Mixed IgE/non-IgE	Combination of IgE-mediated and cell-mediated mechanisms; both antibody and T-cell responses are involved; dendritic cells, macrophages, neutrophils	Both immediate (minutes) and delayed (hours/days) symptoms	Features of both types: e.g., urticaria/angioedema with delayed GI or skin symptoms (eczema)	Atopic dermatitis and eosinophilic esophagitis (both involve IgE and T-cell processes)

3. Clinical History and Physical Examination

The collection of a clinical history is a first step for the diagnosis of suspected food allergies. It helps identify potential IgE-mediated mechanisms and likelihood for true allergic reactions in the decision-making process of which diagnostic tests to perform in order. A detailed clinical history aids in avoiding unnecessary testing, which carries with it several risks and often leads to dietary restrictions that, in turn, can have potential consequences, such as insufficient nutrition and impact on quality of life [21]. Careful history can also aid in the differentiation of an allergic versus non-allergic reaction by documenting the relationship between food and symptom appearance, avoiding misdiagnosis and inappropriate interventions [6,8].

The information regarding the timing and pattern of symptoms are key to assessing suspected food allergies in the clinical history. A rapid onset, typically within minutes to two hours, supports the possibility of an IgE-mediated allergy [6]. In contrast, a delayed presentation of symptoms is often indicative of a non-IgE-mediated reaction and suggests further investigation. It is also valuable to note the food suspected, as well as symptom consistency upon multiple exposures. Gathering these details will help to distinguish food allergies from coincidental or psychosomatic reactions [4,6,8].

Noting the quantity of the food consumed needed to provoke symptoms is crucial, as well as any concomitant factors or triggers. The addition of these details can further classify potential syndromes, such as food-dependent exercise-induced anaphylaxis [4,8,22].

The use of a standardized form or questionnaire can increase reliability of the information collected, allowing clinicians to estimate pre-test probability, improving diagnostic efficiency. This is particularly important when resources are limited [4,6,22].

Based on a detailed allergy history, clinicians may find that an allergic reaction may involve more severe symptoms, and it can aid in risk assessment, to provide guidance on the use of epinephrine [6,8,21].

It is valuable to note any co-existing atopic diseases, such as atopic dermatitis (eczema), asthma, and/or allergic rhinitis, as these conditions may increase the likelihood of a child having food allergies [21]. Having concurrent atopic diseases will also impact the interpretation of diagnostic tests [8,22].

Family history provides additional insight, as patients are at a higher risk for developing allergies if at least one first-degree relative has a diagnosis [21]. If the child or adult patient has previously been exposed to a food allergen in their early years without eliciting a reaction, it is helpful for differentiating a newly developed allergy from a cross-reactivity to a similar food [6,8].

A physical exam provides different levels of information, depending on the presence of acute symptoms. If an allergic reaction is acutely underway, often the provider will see or feel physical manifestations like urticaria (hives), angioedema (swelling under the skin), and/or dyspnea (difficulty breathing) that help to substantiate a diagnosis. Additionally, it supports the immediate decision-making of treatment for the acute phase of the allergic reaction [23]. In between reactions, the physical exam can identify certain inflammatory or atopic signs. Examples can be severe, inadequately controlled atopic dermatitis or physical exam findings suggesting eosinophilic esophagitis or other inflammatory gastrointestinal diseases [6,8].

In some patients, particularly at-risk children or adolescents, the physical examination may yield valuable clues, such as oral mucosa, palate, or tongue changes or dermatographism. These subtle signs may be helpful in interpreting diagnostic tests and predicting the risk for progression to more severe forms of allergic disease [23]. Furthermore, these signs can alert clinicians to potential confounders that are non-allergy or may negatively affect food challenge success [6,12].

History, physical examination findings, and pre-test probability are valuable and complementary when interpreting diagnostic tests. If a patient with a positive SPT (skin prick test) or sIgE (serum specific IgE) lacks a consistent clinical history to that particular food, it is likely to reflect sensitization and not a true allergy [7,22,23].

Clinicians should avoid prescribing food avoidance in the absence of this evidence, as it will have no clinical benefit, yielding a false-positive result. The child may be inappropriately and unnecessarily restricted from eating these foods, and the family may be unnecessarily fearful of introducing them [7,22,23].

In contrast, the likelihood of clinical reactivity increases in a patient with a classic history of a reproducible, immediate-onset allergic reaction, regardless of SPT/sIgE testing, and avoidance should be pursued [7,22,23].

Integrating diagnostic testing and the clinical history allows practitioners to personalize a diagnostic approach to an individual by optimizing their use of resources, diminishing misdiagnosis, and reducing the risks of dietary restriction, improving long-term patient-related outcomes [6,8,22].

4. In Vivo Diagnostic Tests

In vivo diagnostic tests, such as skin-based examinations, provide direct insights into allergic reactivity and play a crucial role in evaluating IgE-mediated food allergies.

These methods compliment the broader diagnostic landscape by providing rapid in-clinic assessments that aid in the refining individual patient diagnoses [6,21,24].

These tests are situated within the overall framework of allergy diagnostics serving as vital tools for distinguishing true allergies from sensitizations and guiding effective management strategies [6,21,24].

4.1. Skin Prick Testing (SPT)

Skin prick testing (SPT) is considered as a first-line and mainstay diagnostic tool for evaluating IgE-mediated food allergy. The test involves introducing a small amount of food allergen extract or fresh food in selected cases, into the superficial layers of the skin typically on the forearm or back and assessing the wheal-and-flare response after approximately 15 min. SPTs are relatively quick, simple, inexpensive and can be performed at any age with a very low risk of systemic reactions [9,24,25].

From a clinical perspective, SPTs are highly sensitive. A negative SPT has a strong negative predictive value, estimated at approximately 90–95%, meaning that a patient with a convincing history but a negative skin test is very unlikely to have an IgE-mediated allergy to the tested food [5]. For this reason, SPT is particularly useful as a rule-out test. In contrast, the specificity of SPT for food allergens is moderate, averaging 50%. A positive result therefore reflects sensitization rather than confirmed clinical allergy, with a positive predictive value of roughly 50%. Many individuals, especially children, may have positive SPC results yet tolerate food without symptoms. Consequently, SPT findings must always be interpreted in the context of a detailed clinical and dietary history, and indiscriminate screening of multiple foods without clinical suspicion is discouraged to avoid identifying sensitizations [24,25].

The size of wheal provides additional diagnostic information. While a wheal diameter of 3 mm or greater than the negative control is generally considered positive, larger wheals correlate with an increased likelihood of true clinical allergy. For certain allergens and populations, decision points associated with high predictive value have been described. For example, a peanut SPT wheal of 8 mm or greater has been associated with a positive predictive value exceeding 95% for clinical reactivity, particularly when supported by a convincing history, and may obviate the need for an immediate oral food challenge [26]. Similarly, egg or milk wheal sized of approximately 6–8 mm in young children have been associated with a very high probability of clinical allergies in some studies. However, these cutoff values vary across studies, allergens, age groups and populations and are not universally applicable. For mildly positive results or equivocal histories, further evaluation with serum specific Age testing or supervised oral food challenges is often required [5].

An important determination of SPT accuracy is the allergen extract used. Commercial extracts are widely available and standardized, but may be suboptimal for certain foods, particularly fruits and vegetables containing labile allergens. In such cases, the prick-to-prick technique using fresh food can improve diagnostic sensitivity and clinical relevance. Studies have shown that homemade or fresh food extracts for some nuts, such as hazelnuts and walnuts can perform as well as commercial extracts and, in some cases, better reflects clinically relevant sensitization [27]. This approach may be particularly valuable in regions where commercial extracts are unavailable or where local dietary exposures differ. However, the prick-to-prick method requires technical expertise and careful interpretation, as variability in food ripeness and preparation can affect reproducibility.

Despite its high sensitivity, SPT has notable limitations. False-positive results are common, especially in pediatric populations, and reliance on SPT alone can lead to over-diagnosis and unnecessary dietary restrictions, which may be nutritionally and psychologically harmful. Sensitization without clinical allergy is relatively frequent, with reported

prevalence of up to 4–10% in early childhood and 2–3% in adults, and even higher rates in infants [25]. Discrepancies between SPT and serum-specific IgE testing are also observed, potentially reflecting differences in assay methodology or the presence of IgE antibodies that do not trigger clinically relevant effector-cell activation *in vivo*.

In summary, SPT is a valuable and highly sensitive screening tool for IgE-mediated food allergy, particularly useful for ruling out allergy when results are negative. A positive SPT supports the possibility of allergy but is not diagnostic on its own due to limited specificity. Larger wheal sizes increase the likelihood of true clinical allergy for certain foods, but interpretation must account for specific allergen extract, including the selective use of fresh food prick-to-prick testing, can further influence diagnostic accuracy. Ultimately, SPT should never be used in isolation, and results must always be integrated with clinical history and when indicated confirmatory testing such as supervised oral food challenges [6,7,24,25,27].

4.2. Intradermal Testing

Intradermal testing (IDT), which involves the injection of a small amount of allergen into the dermis, is not recommended for the routine diagnosis of food allergy. Although IDT can detect immediate IgE-mediated reactions and in some contexts delayed hypersensitivity responses, its role in food allergy evaluation is limited and controversial. Compared with SPT, IDT has higher sensitivity but markedly lower specificity, resulting in a high rate of false-positive results. This reduced specificity largely reflects the detection of clinically irrelevant sensitization rather than true food allergy [24,25].

The increased allergen dose delivered intradermally enhances mast cell activation, histamine release and wheal formation, but this also substantially increases risk of systemic reactions, including anaphylaxis. For this reason, IDT must only be performed by trained professionals with immediate access to emergency equipment and under strict patient monitoring. Children are particularly vulnerable to systemic reactions, further limiting the applicability of IDT in pediatric food allergy assessment [5].

While IDT may be considered as a secondary or tertiary test—for example, in rare cases of severe but poorly documented food allergy when SPT, sIgE, component-resolved diagnostics (CRD) or basophil activation testing (BAT) are unavailable or inconclusive—international guidelines strongly discourage its routine use. The modest gain in sensitivity does not meaningfully improve diagnostic accuracy and does not justify the increased risks. Studies have demonstrated that most positive IDT results do not correlate with clinical reactivity; for instance, only a small portion of children with positive IDT to cow's milk subsequently react during oral food challenges. Consequently, reliance on IDT has been associated with overdiagnosis, unnecessary dietary restrictions, increased healthcare costs, patient anxiety and reduced quality of life [25,27,28].

Additional limitations of IDT include poor reproducibility and inter-center variability, arising from differences in allergen concentration, injection depth, and timing and interpretation of skin responses. These technical inconsistencies further undermine its diagnostic reliability. As a result, clinical practice has shifted away from IDT toward safer and more specific approaches, particularly supervised oral food challenges and advanced *in vitro* tests such as CRG and BAT [25,28].

In contrast to IDT, the atopy patch test (APT) targets delayed (type IV) hypersensitivity reactions rather than IgE-mediated mechanisms. APT assesses cutaneous responses to prolonged allergen exposure and reflects T-cell-mediated inflammation, characterized initially by Th2 cytokines (IL-4 and IL-13) and later by Th1 cytokines such as interferon- γ [7,8,27]. APT has shown utility in selected conditions, including atopic dermatitis, non-IgE-mediated food allergy and eosinophilic esophagitis. However, similar to IDT, APT

suffers from wide variability in sensitivity and specificity, lack of standardized methodology, and inconsistent interpretation. For these reasons, APT is not routinely recommended in standard food allergy diagnostic algorithms [29].

5. In Vitro Diagnostic Tests

Advances in laboratory-based diagnostics are beginning to provide significant insight into the immunological underpinnings of food allergy and more exact risk assessment. This section will review the major in vitro methods used to diagnose food allergies and their applications to the overall diagnostic algorithm discussed earlier [6,22].

5.1. Serum Specific IgE Testing

Serum-specific IgE (sIgE) testing is the most widely available in vitro method for detecting sensitization to food allergens. These tests quantify circulating IgE antibodies directed against specific food antigens and are commonly performed using automated assays such as ImmunoCAP (fluorescent enzyme immunoassay) or older radioallergosorbent test (RAST) platforms [6,30,31]. A positive sIgE result indicates immunologic sensitization, whereas a negative result makes IgE-mediated food allergy unlikely in most cases. Serum sIgE testing is particularly useful if skin testing cannot be performed (for example, if the patient has severe eczema or is taking antihistamines that cannot be stopped) [5]. It is also used as an adjunctive to skin tests, or to quantify the level of sensitization.

Importantly, sensitization does not equate to clinical food allergy. A positive sIgE alone cannot confirm that ingestion of the food will provoke symptoms. Therefore, sIgE results must always be interpreted in conjunction with a detailed and credible clinical history. Failure to make this distinction is one of the most common pitfalls in food allergy management and can lead to overdiagnosis, unnecessary dietary restriction, nutritional deficiencies, and reduced quality of life [7,18].

sIgE testing has relatively high sensitivity and variable specificity, particularly for common allergens such as peanut, cow's milk, and egg. When interpreted alongside a convincing history of immediate allergic symptoms following food ingestion, sIgE testing can strongly support a diagnosis of IgE-mediated food allergy. However, in the absence of such a history, the likelihood of false-positive results increases substantially due to cross-reactivity, detection of clinically irrelevant IgE antibodies, or transient sensitization—especially in infants and young children [24].

Several studies comparing sIgE or skin prick test positivity with double-blind, placebo-controlled food challenges have demonstrated that the prevalence of food sensitization far exceeds the prevalence of true clinical allergy. In pediatric populations, fewer than half of children with positive tests to milk or egg are clinically allergic when formally challenged, and many children outgrow early sensitization without significant symptoms. These findings underscore the necessity of contextualizing laboratory results within the patient's clinical presentation [24,32].

The clinical value of sIgE lies in risk estimation, guiding food introduction or avoidance, monitoring the evolution of sensitization, and determining whether an oral food challenge (OFC) is warranted. For selected foods, population-based cutoff values corresponding to approximately 95% positive predictive value (PPV) for clinical reactivity have been established [20]. Commonly cited pediatric thresholds include approximately 15 kUA/L for peanut, 15 kUA/L for cow's milk, and 7 kUA/L for egg white in children over two years of age, with lower cutoffs applying to infants and toddlers [33].

These cutoff values can, in some cases, allow clinicians to diagnose food allergy without performing an OFC. However, they are derived from specific populations and laboratory assays and may vary by age, geography, and methodology. Crucially, sIgE

concentrations do not reliably predict reaction severity or individual threshold doses. Some patients tolerate foods despite sIgE levels above published cutoffs, while others react at much lower levels. Consequently, reliance on cutoff values alone risks both under- and overdiagnosis [34].

The limitations of sIgE testing are particularly evident in patients with atopic dermatitis, poly-sensitization, or elevated total IgE levels, where false-positive results are common. In such cases, positive sIgE findings may be clinically irrelevant. Conversely, although a negative sIgE has a high negative predictive value, it does not absolutely exclude allergy, and an OFC may still be indicated if clinical suspicion remains high [33].

Given these constraints, major allergy guidelines emphasize that sIgE testing should be targeted to foods suggested by the clinical history. Indiscriminate screening with large food panels is discouraged, as it increases the risk of false-positive results and unnecessary food avoidance [7,18,24].

In conclusion, serum-specific IgE testing remains a fundamental element in the evaluation of suspected food allergy, but its limitations must be clearly recognized. Sensitization detected by sIgE does not equal clinical allergy, and no single cutoff value reliably predicts reaction severity or individual thresholds [7,18,24].

5.2. Component-Resolved Diagnostics

Component-Resolved Diagnostics (CRD) represents a major advancement in the diagnosis and management of food allergy by defining IgE sensitization at the molecular level rather than relying on whole allergen extracts. Traditional serum-specific IgE (sIgE) testing and skin prick tests use extracts that contain mixtures of allergenic and non-allergenic proteins, which can result in false-positive findings and poor risk prediction. In contrast, CRD enables testing for IgE directed against individual allergenic proteins, providing a more precise sensitization profile and improving diagnostic specificity [4,7,18].

CRD is particularly valuable because many foods contain both “major” allergens—such as storage or structural proteins that are resistant to heat and digestion and are strongly associated with systemic or anaphylactic reactions—and “minor” allergens, which are often labile and involved in cross-reactivity with pollens, typically causing mild or localized symptoms. For example, in peanut allergy, IgE sensitization to the storage protein Ara h 2 (and related components such as Ara h 1, Ara h 3, and Ara h 6) is strongly associated with true peanut allergy and severe reactions, whereas IgE to Ara h 8, a Bet v 1 homolog, usually reflects pollen–food syndrome with mild oral symptoms. Similar patterns are observed in other foods, such as hazelnut (Cor a 9 and Cor a 14 versus Cor a 1), cashew (Ana o 3), and wheat (omega-5 gliadin) [4,17,18].

By identifying sensitization to clinically relevant allergenic components, CRD allows for improved risk stratification and more individualized management strategies. Testing against stable storage proteins such as Ara h 2 in peanuts, Cor a 14 in hazelnuts, or Ana o 3 in cashew nuts enables clinicians to identify patients at higher risk of systemic reactions more reliably, facilitating appropriate counseling, emergency action planning, and decisions regarding oral food challenges. Conversely, CRD can reveal sensitization patterns consistent with cross-reactivity rather than true food allergy, helping to avoid overdiagnosis and unnecessary dietary restrictions [4,17,18,22,35].

CRD is also useful for prognostic assessment. In egg and milk allergy, IgE to ovomucoid (Gal d 1) and casein (Bos d 8), respectively, has been associated with more persistent allergy and reduced tolerance to baked forms of these foods. Children sensitized predominantly to these stable proteins are less likely to outgrow their allergy compared with those sensitized only to other components. In addition, CRD can uncover hidden associations and co-sensitizations, such as Pru p 7-mediated systemic peach allergy associated with

cross-reactivity to cypress and Japanese cedar pollen, which is particularly relevant in certain geographic regions [35].

Multiplex CRD platforms, such as ImmunoCAP ISAC, demonstrate increased specificity compared with extract-based testing, with component-specificity exceeding 95% for some allergens, including Gal d 1 in egg and Bos d 5 in milk. This increased specificity is crucial for reducing false-positive diagnoses and minimizing the psychological, social, and nutritional burdens associated with unnecessary food avoidance. However, the sensitivity of CRD varies considerably between different allergenic components. For example, sensitivity for cow's milk allergens ranges from approximately 24% for Bos d 5 to over 80% for Bos d 8, indicating that a negative result for a single component cannot reliably exclude clinical allergy. The lack of universally accepted cutoff values for many components further limits the widespread implementation of CRD in routine clinical practice [35,36].

Despite its advantages, CRD is not required for all patients and should be used selectively, particularly in cases involving peanut and tree nut allergy, multiple sensitizations, or suspected cross-reactivity. Interpretation of CRD results must always be integrated with a thorough clinical history and, when appropriate, oral food challenges, which remain the diagnostic gold standard. Molecular sensitization patterns alone are insufficient to fully predict clinical reactivity, and inaccurate interpretation can lead to misclassification of allergy status. The diagnostic accuracy of CRD may also be influenced by regional differences in diet and environmental exposure, limiting the generalizability of findings across populations [37].

Emerging approaches, such as epitope-based IgE profiling, aim to further refine molecular diagnosis by measuring IgE binding to specific allergen epitopes rather than whole proteins. Proof-of-concept studies using bead-based epitope assays have demonstrated improved diagnostic accuracy for certain food allergies, such as wheat, compared with conventional sIgE testing, highlighting the future potential of epitope-resolved diagnostics [37,38].

In summary, component-resolved diagnostics enhance the precision of food allergy diagnosis by distinguishing clinically relevant sensitizations from benign cross-reactivity. CRD is particularly valuable for peanut and selected tree nut allergies, as well as for prognostic assessment in egg and milk allergy. When applied appropriately and interpreted by experienced clinicians in conjunction with clinical evaluation and complementary tests, CRD can reduce diagnostic uncertainty, minimize unnecessary food avoidance, and support more personalized risk assessment and management strategies.

5.3. Basophil Activation Test

The basophil activation test (BAT) is an ex vivo functional assay that assesses the reactivity of a patient's basophils to specific allergens and has emerged as a highly promising diagnostic tool in IgE-mediated food allergy. Unlike skin prick testing (SPT) or serum-specific IgE (sIgE) measurements, which primarily assess sensitization, BAT evaluates whether allergen-specific IgE is functionally capable of triggering effector cell activation. This allows BAT to differentiate true clinical allergy from asymptomatic sensitization with high diagnostic accuracy [7,25,26].

BAT is performed by incubating a patient's peripheral blood leukocytes with the suspected food allergen. If basophils recognize the allergen via IgE bound to their surface FcεRI receptors, they become activated and upregulate activation markers such as CD63 and/or CD203c. These markers are detected using flow cytometry and correlate with the release of histamine and leukotrienes responsible for allergic symptoms. In this way, BAT directly measures the biological relevance of IgE sensitization rather than its mere presence [7,12,25].

Numerous studies have demonstrated the high diagnostic performance of BAT, with reported sensitivities of approximately 90–91% and specificities around 90%. Positive and negative predictive values are also high, with PPV reported at approximately 81–95% and NPV up to 96–98% in certain settings. This high accuracy makes BAT particularly useful in complex diagnostic scenarios, such as differentiating peanut allergy from peanut sensitization, resolving “gray-zone” IgE results, or evaluating patients with multiple food sensitizations. In several clinical trials, BAT has provided diagnostic clarity when conventional tests such as SPT or sIgE were inconclusive or misleading [7,25,39].

A major clinical advantage of BAT is its ability to reduce reliance on oral food challenges (OFCs), which remain the diagnostic gold standard but are resource-intensive, costly, and carry a risk of inducing severe allergic reactions. By reliably confirming or excluding food allergy, BAT can streamline diagnostic workflows, reserving OFCs for selected uncertain cases. This is particularly beneficial in pediatric populations, where unnecessary food avoidance can negatively affect nutrition, growth, and quality of life. BAT has also been shown to identify tolerance to previously allergenic foods, such as baked egg or milk, making it useful in patients suspected of having outgrown these allergies [7,26,39].

Beyond diagnosis, the degree of basophil activation has been correlated with clinical reaction severity observed during oral food challenges. Strong basophil activation at low allergen doses has been associated with an increased risk of severe reactions, enabling improved risk stratification, emergency planning, and more informed decisions regarding food challenges. Furthermore, BAT can monitor changes in basophil reactivity over time, suggesting a role in assessing disease progression, remission, and response to immunomodulatory treatments such as oral immunotherapy or biologic agents.

Current European Academy of Allergy and Clinical Immunology (EAACI) guidelines acknowledge BAT as a second-line diagnostic test, recommended when clinical history and standard testing do not yield conclusive results. Its use has been proposed not only for diagnostic confirmation but also for monitoring allergy evolution and selecting candidates for immunotherapeutic interventions, particularly in patients with complex or multiple food allergies.

Despite these strengths, BAT has practical limitations that restrict its widespread implementation. The test requires fresh blood samples to preserve basophil viability and must be processed within 24 h, necessitating access to on-site flow cytometry facilities and trained personnel. The assay is technically demanding, labor-intensive, and more costly than conventional tests, and variability in protocols and interpretation remains a challenge, highlighting the need for further standardization. Although BAT can be performed with relatively small blood volumes, it still requires more hands-on time than SPT or routine sIgE testing [25,39,40].

In parallel, the mast cell activation test (MAT) has emerged as a newer functional diagnostic approach. MAT uses primary human mast cells that are sensitized *in vitro* with patient serum or plasma and then exposed to allergens. Allergen-specific activation and degranulation are assessed by measuring surface markers such as CD63 and CD107a, as well as functional readouts including prostaglandin D₂ production and β -hexosaminidase release. MAT has demonstrated very high diagnostic performance, with reported sensitivities of up to 97% and specificities of approximately 92%, in some cases exceeding those of BAT [40].

An important advantage of MAT is that it uses patient serum, which can be frozen and stored, overcoming the logistical constraints associated with fresh blood requirements for BAT. Human mast cells also show greater stability and reproducibility, making MAT an attractive tool for studying effector cell mechanisms and allergen reactivity. More recently, novel approaches such as the Hoxb8 mast cell activation test have shown promis-

ing diagnostic precision for peanut allergy and may help classify patients who are BAT nonresponders, further improving decision-making in difficult cases [7,25,26,39–41].

In summary, the basophil activation test is one of the most accurate laboratory tools currently available for diagnosing IgE-mediated food allergy, offering high sensitivity and specificity and a unique ability to distinguish true allergy from benign sensitization. When used alongside clinical history, component-resolved diagnostics, and other conventional tests, BAT can significantly improve diagnostic precision and reduce unnecessary oral food challenges. While practical limitations currently confine its use to specialized centers, continued validation, standardization, and technological advances may facilitate broader clinical adoption. The development of complementary assays such as MAT further underscores the growing role of functional cellular testing in the future of food allergy diagnostics.

5.4. Mast Cell Activation Test (MAT)

The mast cell activation test (MAT) is a novel and innovative diagnostic assay conceptually similar to the basophil activation test (BAT), but it utilizes mast cells rather than basophils as the effector cells. Mast cells are tissue-resident immune cells found in the skin, gastrointestinal tract, and other organs and are the principal mediators of IgE-mediated allergic reactions. In MAT, laboratory-cultured human mast cells are passively sensitized with patient serum containing allergen-specific IgE antibodies and subsequently exposed to the suspected allergen. Allergen-induced mast cell activation is then quantified by assessing surface expression of activation markers, such as CD63, or by measuring released mediators, including β -hexosaminidase, typically using flow cytometry or functional assays [40,42].

The development of MAT addresses several limitations associated with BAT and other diagnostic methods. Because mast cells more closely reflect the effector cells involved in tissue-level allergic reactions, MAT may better mirror *in vivo* responses. Additionally, the use of standardized mast cell lines or cultured cells reduces donor-dependent variability, which is a known limitation of basophil-based assays. Since sensitization is achieved through passive transfer of patient IgE via serum, MAT can be performed even when basophil function is suppressed by medications, and serum samples can be stored or transported more easily than fresh whole blood [40,42,43].

Early studies suggest that MAT demonstrates excellent diagnostic accuracy. A proof-of-concept study using double-blind placebo-controlled peanut challenges showed that MAT was superior to conventional diagnostic tests—including skin prick testing, serum-specific IgE, and BAT—in distinguishing true peanut allergy from asymptomatic sensitization. The degree of mast cell degranulation observed in MAT was also found to correlate with clinical outcomes, indicating potential utility not only in diagnosis but also in risk stratification [42,43].

An additional practical advantage of MAT is its logistical flexibility. Unlike BAT, which requires fresh blood samples and rapid processing, MAT relies on stable serum samples and laboratory-prepared mast cells that can be cryopreserved or centralized, making large-scale implementation more feasible in the future. This approach could allow patient samples to be collected locally and analyzed in specialized laboratories [40,42,43].

Despite its promise, MAT remains primarily a research tool and is not yet widely available in routine clinical practice. The assay requires advanced laboratory infrastructure and technical expertise to culture and sensitize mast cells, and further standardization across mast cell lines, protocols, and allergens is necessary. Additionally, cost-effectiveness and scalability will need to be evaluated as the technology matures [40,42,43].

In summary, the mast cell activation test represents a next-generation approach to food allergy diagnostics, offering a controlled and physiologically relevant *in vitro* model

of allergic reactions. Although still under development, early evidence indicates that MAT may outperform existing diagnostic methods in distinguishing true food allergy from sensitization. With continued validation and standardization, MAT has strong potential to become a valuable clinical tool and to further reduce reliance on oral food challenges, enhancing both diagnostic accuracy and patient safety.

5.5. Atopy Patch Test (APT)

The atopy patch test (APT) is a diagnostic tool designed to identify non-IgE-mediated or mixed IgE/cell-mediated immune reactions to foods. In this procedure, a suspected food allergen—either fresh or in powdered form, commonly prepared in petrolatum or saline—is applied to the skin under an occlusive patch, typically on the patient's back, and left in place for approximately 48 h. The test site is then evaluated at 48 and 72 h for eczematous changes such as erythema, infiltration, or papules, which indicate a delayed-type hypersensitivity response [29,44].

APT has primarily been investigated in non-IgE-mediated gastrointestinal food allergies, including food protein-induced allergic proctocolitis, food protein-induced enterocolitis syndrome (FPIES), food-induced enteropathy, and eosinophilic gastrointestinal disorders. It has also been studied in selected patients with atopic dermatitis in whom food triggers are suspected. In these conditions, conventional immediate-type diagnostic tests, such as skin prick testing and serum-specific IgE measurements, are often negative or insufficiently predictive. The rationale for APT is to reproduce a delayed cutaneous immune response that may reflect underlying gastrointestinal inflammation following food ingestion [29,45,46].

The diagnostic accuracy of APT remains controversial; however, recent evidence suggests that it demonstrates high specificity in certain clinical contexts. A 2023 systematic review and meta-analysis evaluating children with non-IgE-mediated gastrointestinal food allergies reported pooled specificity values of approximately 94–96%, indicating a very low false-positive rate. Notably, in cases of cow's milk allergy presenting with delayed gastrointestinal symptoms, APT exhibited excellent specificity (~96%) and strong overall diagnostic accuracy, with a pooled area under the curve of approximately 0.93. These findings suggest that a positive APT result, particularly when aligned with a compatible clinical history, is highly predictive of a true causative food allergen [29,44–46].

Similarly, studies examining food-induced gastrointestinal motility disorders have shown that APT may effectively identify offending foods with high specificity, potentially reducing the need for multiple empirical elimination diets. However, the sensitivity of APT is consistently reported as low to moderate. Meta-analytic data indicate an overall sensitivity of approximately 46–52%, meaning that a negative APT result does not reliably exclude non-IgE-mediated food allergy. Sensitivity varies widely across studies and disease phenotypes, with some reports documenting rates as low as 20%. Consequently, negative APT results must be interpreted cautiously and in conjunction with clinical history and, when indicated, oral food challenges [29,44–46].

A major limitation of APT is the lack of standardized methodology. There are no universally accepted commercial reagents, preparation techniques, allergen concentrations, or scoring criteria. Variability in test materials and interpretation limits reproducibility and comparability across centers. In addition, the assessment of mild eczematous reactions can be subjective, further reducing reliability. As a result, APT is not routinely recommended as part of the standard diagnostic work-up for food allergy, and most national and international guidelines restrict its use to research settings or selected clinical scenarios [29,44,45].

In specific conditions such as eosinophilic esophagitis, APT has been incorporated into some diagnostic protocols to help guide elimination diets, particularly in pediatric

populations. However, outcomes have been inconsistent, and the test has not demonstrated sufficient reliability to replace established diagnostic approaches [29,44–46].

In summary, the atopy patch test may serve as a useful adjunctive tool in the evaluation of non-IgE-mediated food allergies when high specificity is desired. A positive APT result provides strong supportive evidence that a particular food is responsible for delayed clinical symptoms. Nevertheless, its low sensitivity, methodological variability, and limited standardization restrict its role in routine clinical practice. When used, APT findings should be interpreted within the broader clinical context and, whenever possible, confirmed by medically supervised oral food challenges, which remain the definitive diagnostic standard even in non-IgE-mediated food allergy.

6. Oral Food Challenge as Gold Standard

Oral food challenges (OFCs) are universally regarded as the gold standard for the diagnosis of food allergy because they provide direct evidence of clinical reactivity. Unlike in vivo and in vitro diagnostic tests—such as skin prick testing (SPT), serum-specific IgE (sIgE), component-resolved diagnostics (CRD), or cellular assays—OFCs do not measure surrogate markers of sensitization or effector cell activation. Instead, they directly assess whether ingestion of a suspected food triggers objective allergic symptoms, thereby minimizing diagnostic uncertainty and definitively clarifying a patient's allergy status [12,24,47].

During an OFC, the patient ingests the suspected allergen in gradually increasing doses under close medical supervision, with careful monitoring for symptoms. OFCs can be conducted as open challenges, single-blind challenges, or double-blind placebo-controlled food challenges (DBPCFCs). DBPCFCs are considered the most rigorous method, particularly in research settings, as they eliminate patient and observer bias. In routine clinical practice, however, open or single-blind challenges are often sufficient when standardized protocols and objective symptom criteria are applied [12,24,47,48].

A major strength of OFCs is their ability to determine whether sensitization detected by surrogate tests translates into true clinical allergy. Asymptomatic sensitization is common, and many patients with positive SPT or sIgE results can tolerate the implicated food without symptoms. OFCs prevent unnecessary dietary restrictions by distinguishing clinically irrelevant sensitization from true allergy, thereby avoiding the negative psychological, social, and nutritional consequences of overdiagnosis. This is particularly important in children, where overly restrictive diets can impair growth, bone health, and quality of life [12,47–49].

In addition to confirming or excluding allergy, OFCs can determine the minimum eliciting dose of an allergen. This information is valuable for patient counseling and risk assessment, as individuals with high reaction thresholds may not require strict avoidance and may safely tolerate trace amounts of an allergen, such as those present in processed foods. OFCs can also identify delayed or mixed allergic reactions that are difficult to diagnose using surrogate tests, offering a comprehensive view of symptom onset, progression, and phenotype [48,49].

OFCs play a central role in allergy diagnostic algorithms and are considered the cornerstone of evidence-based management of IgE-mediated food allergy. They are particularly useful when clinical history is unclear, when test results are borderline or conflicting, or when patients show sensitization to multiple foods of uncertain clinical relevance. OFCs are also essential for monitoring allergy resolution, especially in childhood, as many children outgrow allergies to common foods such as milk or egg. Periodic challenges allow for timely and safe reintroduction of foods, improving nutritional intake and overall quality of

life. OFCs are similarly used to assess tolerance to baked forms of milk or egg, which can substantially broaden the diet for allergic patients [47–49].

Despite their unmatched diagnostic accuracy, OFCs are resource-intensive and require specialized facilities, trained allergy professionals, and prolonged observation periods. They carry an inherent risk of inducing allergic reactions, including anaphylaxis, although severe outcomes are rare when challenges are performed according to standardized safety protocols. Because of these risks and logistical demands, OFCs are typically reserved for situations in which other diagnostic tools do not provide a clear answer or when confirming resolution of a known allergy [24,48,49].

Strict adherence to standardized protocols is essential to ensure patient safety and maintain confidence in the procedure. OFCs must be conducted with immediate access to emergency medications, including epinephrine, and by teams experienced in recognizing and managing allergic reactions. Careful patient selection is critical: factors such as reaction history, pre-test probability, comorbid conditions, and the patient's ability to manage potential reactions should all be considered before proceeding. Tailoring the decision to perform an OFC to the individual patient helps balance diagnostic benefit against procedural risk [12,24,47–50].

Although laboratory and surrogate tests are commonly used to assess risk before an OFC, no single biomarker reliably predicts whether a reaction will occur or how severe it will be. Elevated sIgE levels, positive SPTs, or positive basophil activation tests (BAT) may increase the likelihood of a reaction but have limited positive predictive value for reaction severity. Consequently, in-person medical supervision remains essential for all OFCs. Surrogate tests should be viewed as tools to estimate probability and guide preparation, not as substitutes for direct challenge testing [47–49].

In summary, oral food challenges remain the definitive diagnostic test for food allergy and the final arbiter when other results are inconclusive. While they carry risks and require substantial resources, OFCs are indispensable for ensuring accurate diagnosis, preventing unnecessary food avoidance, and safely reintroducing foods when tolerance has developed. Advances in laboratory diagnostics aim to make OFCs more selective and better targeted, but no existing test can fully replace the clinical certainty provided by a properly conducted food challenge [12,24,50].

Artificial intelligence approaches are increasingly being explored to integrate clinical history with panels of diagnostic tests (e.g., sIgE, CRD, BAT/MAT) and thereby reduce uncertainty before proceeding to oral food challenges [39,41].

In pediatric peanut allergy cohorts, machine-learning models have demonstrated potential to predict oral food challenge outcomes, suggesting a future role for decision-support tools in clinical practice [40].

7. Conclusions

The primary aim of this research was to evaluate current methods for diagnosing food allergies and to compare traditional diagnostic approaches with emerging molecular and cellular techniques. The overarching objectives were to improve diagnostic efficiency and certainty, reduce misdiagnosis, enhance food safety, and ultimately improve patient outcomes and quality of life. A central challenge addressed throughout this work was the differentiation of true food allergy from asymptomatic sensitization, a distinction that remains a major source of diagnostic uncertainty in clinical practice.

The findings of this thesis demonstrate that accurate diagnosis of food allergy requires a stepwise, individualized approach that integrates a detailed clinical history and thorough physical examination with selectively applied in vivo and in vitro testing. Clinical history remains the cornerstone of assessment, as it allows clinicians to interpret diagnostic test

results within the appropriate clinical context and assess the likelihood of true allergic reactivity. Skin prick testing (SPT) and serum-specific IgE (sIgE) measurements are essential first-line tools due to their high sensitivity, making them particularly useful for ruling out IgE-mediated food allergy when results are negative. However, positive results are common and frequently reflect sensitization rather than true clinical allergy, resulting in low specificity and a high rate of false-positive diagnoses.

The use of established high-predictive-value cutoff thresholds for SPT wheal size or sIgE levels can improve diagnostic specificity in selected cases, allowing clinicians to confirm a diagnosis without proceeding to an oral food challenge (OFC). Nevertheless, variability in cutoff values between allergens, populations, and geographic regions limits their universal applicability. Cross-reactivity and the inability of sIgE levels to reliably predict clinical reactivity further underscore the need for more refined diagnostic tools.

Novel diagnostic modalities, including component-resolved diagnostics (CRD) and cellular assays such as the basophil activation test (BAT), represent significant advances in improving diagnostic precision. CRD allows for the identification of IgE sensitization to specific allergenic proteins, helping to distinguish clinically relevant sensitization from benign cross-reactivity, as exemplified by markers such as Ara h 2 in peanut allergy. BAT provides functional evidence of allergic potential by assessing basophil activation in response to allergens and has demonstrated high sensitivity and specificity in differentiating true food allergy from tolerance, particularly in cases of polysensitization or ambiguous clinical history. Experimental approaches such as the mast cell activation test (MAT) may further enhance diagnostic accuracy in the future.

Despite these advances, the oral food challenge remains the gold standard for confirming food allergy and tolerance. OFCs provide direct evidence of clinical reactivity and bypass the inherent limitations of surrogate diagnostic tests. When performed in controlled settings by experienced clinicians, OFCs allow for precise diagnosis, determination of reaction thresholds, assessment of delayed or mixed phenotypes, and safe reintroduction of foods in patients who have developed tolerance. This stepwise challenge approach minimizes unnecessary dietary elimination, reduces the risk of nutritional deficiencies and psychosocial burden, and improves overall quality of life for patients and their families.

This thesis supports current clinical guidelines emphasizing that food allergies are both underdiagnosed and overtreated, largely due to overreliance on sensitization-based tests without adequate clinical correlation. An individualized diagnostic pathway for IgE-mediated food allergy is essential to minimize unnecessary food avoidance while ensuring that truly allergic patients are correctly identified and protected. Integrating molecular and cellular diagnostic results into clinical decision-making allows for more targeted risk assessment and personalized management strategies.

Nevertheless, several important limitations persist. Many existing studies are limited by small sample sizes, variable prevalence rates, and inconsistent diagnostic thresholds. Accessibility remains a major barrier to the widespread implementation of advanced diagnostics such as CRD and BAT, as these tests are costly, require specialized laboratory infrastructure, and demand expert interpretation. Furthermore, findings from *in vitro* studies cannot always be extrapolated directly to clinical outcomes, as complex host, environmental, and genetic factors influence allergic responses. Geographic variation in allergen exposure, dietary practices, and genetic background further limits the generalizability of diagnostic algorithms across populations. In addition, OFCs themselves are resource-intensive and variably standardized across centers.

Future research should focus on the identification and validation of novel biomarkers and clinically meaningful cutoff values across diverse allergens, age groups, and populations. Large, multicenter studies are essential for standardization, validation, and global

applicability of diagnostic tools. Advances in “omics” technologies may provide deeper insights into immune mechanisms underlying food allergy and tolerance, while machine learning and artificial intelligence hold promise for improving predictive accuracy and integrating complex diagnostic data. Continuous validation and adaptation of diagnostic policies will be necessary as new technologies emerge.

Based on the current evidence and available resources, a recommended diagnostic pathway includes careful clinical history assessment, followed by targeted use of SPT and/or sIgE testing. CRD and BAT should be considered in complex or ambiguous cases. OFCs should be reserved for situations in which diagnostic uncertainty persists or tolerance is suspected and must be performed by experienced healthcare providers in controlled settings. Management strategies should then be individualized, ranging from dietary modification and symptomatic treatment to emergency preparedness with epinephrine autoinjectors in confirmed cases of food allergy.

In conclusion, the findings of this thesis help bridge critical gaps in food allergy diagnosis by emphasizing a balanced, evidence-based, and patient-centered approach. By optimizing the use of available diagnostic tools and integrating emerging technologies thoughtfully, clinicians can improve diagnostic accuracy, reduce unnecessary interventions, enhance patient safety, and ultimately improve health outcomes and quality of life for individuals affected by food allergies.

A summary of diagnostic methods used in the diagnosis of food allergy is presented in Table 2.

Table 2. Diagnostic Methods for Food Allergy [5,24–50].

Test	Mechanism	Advantages	Limitations	Clinical Utility
Skin Prick Test (SPT)	Allergen introduced via skin pricks; cross-linking of food-specific IgE on mast cells triggers degranulation and wheal formation	High sensitivity (~90%); rapid and low-cost; multiple allergens can be tested simultaneously	Low specificity (~50%); false positives possible; results can be suppressed by antihistamines or affected by dermatographism	Initial screening for IgE-mediated allergy; positive SPT guides further testing or elimination diets in the context of history
Serum specific IgE (sIgE) test	In vitro immunoassay (e.g., ImmunoCAP) quantifying specific IgE levels in patient serum	Unaffected by skin condition or medications (e.g., antihistamines) quantitative and standardized; can test many allergens from a single blood sample	Lower specificity; positive result indicates sensitization not always clinical allergy; cross-reactivity can confound results; variable cutoff values	Identifies sensitization profile; complements SPT and history to confirm suspected allergies; useful when SPT is contraindicated
Component-Resolved Diagnostics (CRD)	Measures IgE binding to purified allergen components (individual proteins) rather than whole extracts	High specificity; distinguishes genuine sensitization from cross-reactivity; can improve prediction of clinical risk (e.g., peanut Ara h2)	Limited availability; requires specialized technology (e.g., microarrays); high cost; complex interpretation; still mainly research-use in routine practice	Useful in polysensitized patients or unclear cases; refines diagnosis by highlighting clinically relevant allergen components; aids risk stratification and dietary advice
Basophil Activation Test (BAT)	Flow-cytometry assay that measures basophil activation markers (CD63/CD203c) after allergen exposure, indicating an IgE-mediated response	Very high sensitivity and specificity; reliably distinguishes true allergy from asymptomatic sensitization; can rank severity of sensitization	Requires fresh blood (processed within ~24 h); specialized lab equipment and expertise; high cost; not widely available	Reserved for complex or ambiguous cases (e.g., multiple sensitizations, unclear history); helps decide need for oral challenge and avoids unnecessary challenges when negative
Oral Food Challenge (OFC)	Medically supervised ingestion of suspected food with incremental dosing, monitoring for allergic reaction	Gold-standard test; directly confirms or excludes clinical allergy; establishes reaction threshold; detects delayed or mixed reactions	Time- and resource-intensive; risk of inducing severe reaction or anaphylaxis; requires appropriate facilities and emergency measures	Definitive diagnosis of food allergy; informs management (avoidance/tolerance); used when history or other tests are inconclusive to ensure accurate diagnosis

Table 2. Cont.

Test	Mechanism	Advantages	Limitations	Clinical Utility
Atopy Patch Test (APT)	It involves triggering a delayed, T-cell-mediated skin inflammatory response after local exposure to a food allergen, reflecting non-IgE or mixed immune mechanisms	High specificity in identifying causative foods in non-IgE-mediated or mixed food allergies, providing strong supportive evidence when positive	Lack of standardization	Supporting the diagnosis of non-IgE-mediated or mixed food allergies, particularly when conventional IgE-based tests are negative, helping guide elimination diets and identify potential triggers in conditions like atopic dermatitis or gastrointestinal food allergies
Mast Cell Activation Test (MAT)	It detects allergen-induced IgE-mediated reactions by measuring degranulation markers, such as CD63 or CD203c, on patient-derived basophils or mast cells in vitro	It can safely assess IgE-mediated allergic responses in vitro, avoiding the risks of triggering a clinical reaction during testing	Limited availability, high cost, technical complexity, and variable sensitivity depending on allergen type and patient IgE levels	Diagnosing IgE-mediated allergies—especially when conventional tests (skin prick or serum-specific IgE) are inconclusive or when oral food challenges pose a high risk—by providing a safer in vitro assessment of mast cell activation

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