Advantages and Limitations of Laboratory Allergy Diagnostics

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Summary

Laboratory diagnostics of allergies has evolved from the initial determination of eosinophil granulocytes count in the blood to more sophisticated, sensitive and specific methods. Advances in diagnostics was the discovery of immunoglobulin E (IgE), including the determination of the concentration of total IgE (tIgE), and then the concentration of specific IgE (sIgE) and eosinophil cationic protein (ECP). At the beginning of the 21st century, more advanced methods began to be used, such as component resolved diagnostics, in which the concentration of sIgE to individual allergen components/molecules is measured, as well as testing the activation of basophil granulocytes ex vivo. Each of the mentioned methods has its diagnostic scope, that is, specific advantages, but also limitations as well. Determination of tIgE is used to screen people with atopy; sIgE indicates the allergens that led to sensitization; component resolved diagnosis detects genuine and cross-reactive allergen molecules. By determining ECP and BAT, the functional ability of eosinophilic and basophilic granulocytes is examined. The described methods, especially component resolved diagnosis and BAT, significantly help the physician, based on the history, clinical picture and other diagnostic procedures in vivo, to make a diagnosis and apply appropriate therapy. Today, the era of the application of “omics” technologies (e.g. genomics and proteomics) has begun, which, together with artificial intelligence for data analysis, lead to personalized allergy diagnostics.

Keywords: laboratory diagnostics, IgE, component resolved diagnostics, omics technologies, artificial intelligence

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1. Introduction

Although, from a historical perspective, the beginnings of laboratory diagnostics of allergies can be attributed to Paul Ehrlich, who in 1879 introduced differential staining and microscopic examination of eosinophilic granulocytes (1), the development of specific laboratory diagnostics of allergies is more closely associated with the discovery of immunoglobulin E (IgE). IgE was discovered in 1966 and described one year later by Kimishige and Teruko Ishizaka (2).

Initially, the concentration of total IgE (tIgE) was measured, and from 1974 onwards, the determination of allergen-specific IgE (sIgE) against allergen extracts was introduced. With the advancement of immunochemical techniques, methods for IgE determination (both tIgE and sIgE) have evolved from radioisotopic assays—such as RIST (radioimmunosorbent test) and RAST (radioallergosorbent test)—to enzyme immunoassays (EIA), fluoroenzyme immunoassays (FEIA), and chemiluminescent immunoassays (CLIA) (Figure 1).

Since 1999, component-resolved (molecular) diagnostics have been used in specialized clinical laboratories to determine concentrations of specific IgE (sIgE) antibodies to individual allergenic molecules. Since 2009, this approach has also been applied as a multiparametric method enabling the simultaneous determination of sIgE to a larger number of allergenic molecules (3). The basophil activation test (BAT), introduced into laboratory practice in 2005, monitors the activation and degranulation of basophilic granulocytes under *ex vivo* conditions (4). Parameters of laboratory allergy diagnostics can be used as diagnostic, prognostic, predictive, and/or pharmacodynamic biomarkers.[1]

In the context of the rapid development of laboratory medicine, recent years have seen the increasing application of methods from the field of omics (genomics, proteomics, epigenomics,

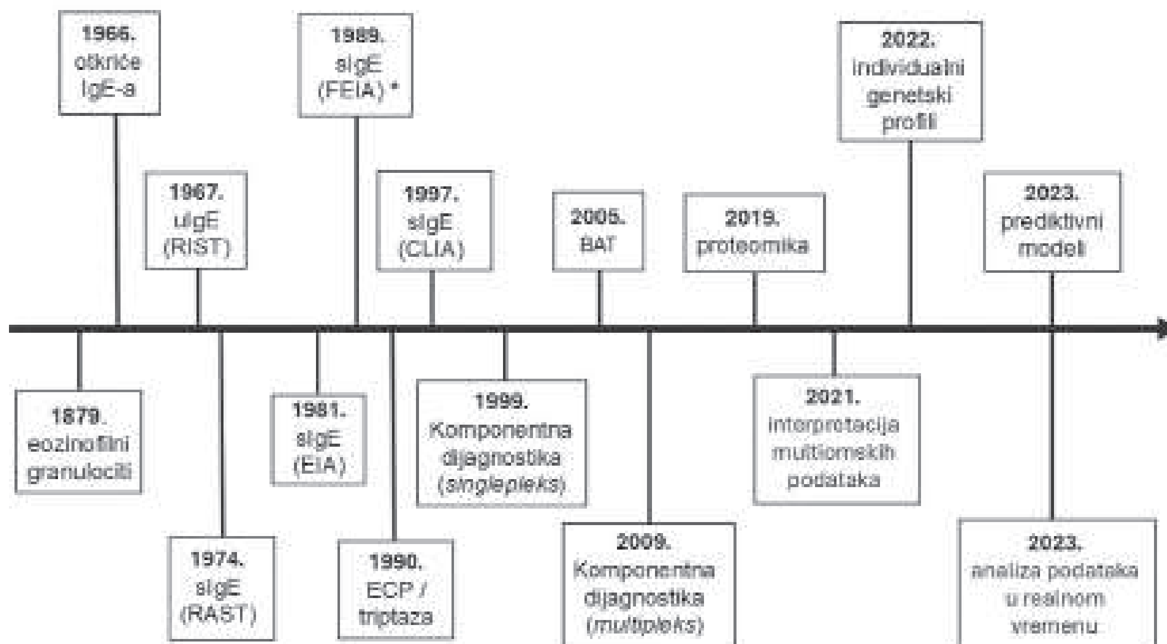

Figure 1. Timeline of laboratory diagnostics of allergies.
BAT – basophil activation test; CLIA – chemiluminescent immunoassay; ECP – eosinophil cationic protein; EIA – enzyme immunoassay; FEIA – fluoroenzyme immunoassay; RIST – radioimmunosorbent test; RAST – radioallergosorbent test; sIgE – specific immunoglobulin E.
*gold standard.

1 Diagnostic biomarkers indicate the presence of a disease. Prognostic biomarkers predict disease progression or outcome regardless of therapy. Predictive biomarkers indicate the likelihood of response to a specific treatment. Diagnostic biomarkers reflect the pharmacological activity of a drug, demonstrate whether a drug effect has occurred, and enable real-time dose adjustment.

transcriptomics, and metabolomics)[2], as well as artificial intelligence models (e.g., machine learning, deep learning, neural networks, statistical clustering, and others).[3] A common characteristic of these approaches is the analysis of vast amounts of laboratory and clinical data. In allergology—where genetics, immunology, biochemistry, and clinical and laboratory medicine intersect—it is essential to understand the capabilities and limitations of laboratory allergy diagnostics in order to apply the obtained results rationally in the diagnosis and treatment of each individual patient.

2. Eosinophils and Eosinophil Cationic Protein

In individuals with allergic diseases, eosinophilia of varying severity is commonly present and reflects the migration of eosinophils from the bone marrow into the circulation and subsequently into tissues affected by allergic inflammation. In affected tissues, eosinophils accumulate within 24 hours after exposure to the causative allergen. Therefore, during the first few hours following the onset of an acute allergic reaction, peripheral blood eosinophil counts may remain within the normal range until newly produced eosinophils are released from the bone marrow.

An increased number of eosinophils in nasal mucosal smears, sputum, or induced sputum represents a biomarker of both allergic inflammation and its severity (5). Determination of eosinophil counts is simple, widely available, inexpensive, and does not require additional equipment. However, its limitations include low specificity, the inability to reflect eosinophil activation or degranulation, and high intraindividual variability.

In addition to circulating eosinophil counts, measurement of eosinophil cationic protein (ECP)—a cytotoxic product released from the large granules of eosinophils—is used to assess their functional activity. ECP reflects the intensity of allergic inflammation more accurately than peripheral eosinophilia. It should be noted, however, that the highest ECP concentrations are observed in metazoan parasitic infections. Clinically, ECP is particularly useful for adjusting corticosteroid therapy in asthma and for assessing the intensity of allergic inflammation in atopic dermatitis (6). Unlike eosinophil counts, ECP reflects eosinophil activation.

Routine clinical practice has demonstrated that intraindividual circadian and seasonal variations in ECP concentrations represent important limitations of its measurement (7). To minimize preanalytical interferences—either clinical (eosinophilia caused by parasitic infections, viral infections, corticosteroid therapy, initiation of allergen-specific immunotherapy [AIT]) or laboratory-related (sample temperature, clotting time, sample type [serum rather than plasma], hemolysis, lipemia)—blood sampling and sample processing must be standardized. Under these conditions, ECP may be applied as a prognostic, predictive, and pharmacodynamic biomarker.

3. Total IgE

Measurement of tIgE concentration is used as an approximate screening method for identifying individuals with atopy (8). An advantage of this

2 Genomics is an interdisciplinary field of molecular biology focused on the structure, function, evolution, mapping, and editing of the genome. Transcriptomics refers to the study of the transcriptome, i.e. the complete set of RNA transcripts of an organism. Proteomics studies proteins, particularly the proteome, which comprises the entire set of proteins produced by a cell or organism. Metabolomics investigates small molecules or metabolites within biological systems such as cells, tissues, or biological fluids. Epigenomics examines the influence of environmental factors on gene regulation.

3 Artificial intelligence (AI) is the simulation of human intelligence processes by machines or computer systems. Machine learning is a subfield of AI focused on developing algorithms that enable systems to learn from data and make predictions without explicit human intervention. Deep learning is a subdomain of machine learning that applies artificial neural networks to analyze large datasets. Neural networks are deep learning techniques designed to resemble the structure of the human brain. Clustering is a machine learning method that automatically groups data into clusters based on similarity.

method is that it is quantitative and standardized according to the World Health Organization reference standard. When measuring serum concentrations, only free IgE is assessed, not IgE bound to FcεRI receptors on basophils or mast cells.

Serum tIgE concentrations in healthy individuals range from very low levels in infancy (< 20 kIU/L) to substantially higher values after the age of ten (< 105 kIU/L) and in adulthood (< 120 kIU/L). Approximately 5% of non-atopic individuals have tIgE concentrations above the upper reference limit, while about 10% of atopic individuals have tIgE values within the reference range (9). The relative increase in IgE concentration is comparable in atopic and non-atopic individuals. Therefore, tIgE values within the reference range must be interpreted with caution, particularly due to variations caused by seasonal exposure to specific allergens.

In addition to age (8, 9) and geographical origin, reference intervals for tIgE depend on the analytical method used, such as turbidimetric assays, FEIA (the reference method for tIgE), and CLIA (10). Consequently, for longitudinal monitoring in the same individual, tIgE concentrations should be measured using the same analytical method.

If there is a justified clinical suspicion of allergy, sIgE concentrations to suspected causal allergens should be determined, guided by medical history, clinical presentation, and skin prick test results. The clinical utility of tIgE measurement includes screening for atopy, assessment of allergy risk, and estimation of symptom severity. It should be noted that in sensitization to seasonal inhalant allergens, peak tIgE concentrations occur four to six weeks after the peak pollen exposure, with the lowest levels observed before the pollen season. After the age of 50, tIgE concentrations gradually decline.

Because tIgE may be nonspecifically elevated in various other conditions (e.g. parasitic infections or malignancies), its diagnostic value depends on the overall clinical and laboratory context.

4. Specific IgE

Measurement of sIgE concentrations is performed based on the patient's medical history, clinical presentation, elevated tIgE, and skin test results. The aim is to identify the allergen to which the patient is sensitized; therefore, sIgE can be considered a diagnostic biomarker of sensitization and evidence of atopy. For this purpose, sIgE concentrations are determined against allergens (allergen extracts) such as house dust mites (e.g. *Dermatophagoides pteronyssinus*, Der p; *Dermatophagoides farinae*, Der f), various tree pollens (e.g. birch – *Betula verrucosa*, Bet v; hazel – *Corylus avellana*, Cor a), weeds (e.g. ragweed – *Ambrosia artemisiifolia*, Amb a; mugwort – *Artemisia vulgaris*, Art v), grasses (e.g. orchard grass – *Dactylis glomerata*, Dac g; Kentucky bluegrass – *Poa pratensis*, Poa p), animal epithelia (e.g. cat – *Felis*, Fel d; dog – *Canis*, Can f), molds (e.g. *Aspergillus fumigatus*, Asp f), foods (e.g. peanut – *Arachis hypogaea*, Ara h; salmon – *Salmo salar*, Sal s), and others.

A positive sIgE result indicates sensitization but not necessarily the cause of an allergic reaction (11). When elevated sIgE concentrations correspond with allergic symptoms and positive skin prick test results, the identified allergen is likely to be clinically relevant. However, results of skin testing and sIgE measurement are not always concordant. This discrepancy arises because skin testing reflects local mast cell activation and immediate cutaneous reactivity, whereas sIgE reflects a systemic immune response. Moreover, sIgE concentrations depend on the time elapsed since exposure to the causative allergen, such as seasonal allergens, drugs (e.g. penicillin), or Hymenoptera venom (bee, wasp, hornet).

Therefore, when measuring sIgE to seasonal inhalant allergens or food allergens, blood sampling should be performed between 2–3 weeks and up to 6 months after the onset of pollen exposure or food ingestion, when sIgE concentrations are highest (5). Most studies have shown that skin prick testing has higher sensitivity than sIgE testing, whereas diagnostic specificity is comparable for both methods (12).

Antihistamine therapy does not interfere with sIgE determination. Analytical methods reliably measure sIgE concentrations within the range of 0.35–100.0 kUA/L. Moderately elevated sIgE levels (> 3.51 kUA/L; class 3) generally require additional interpretation in the context of clinical data and skin test results. Significantly elevated concentrations are defined as values ≥ 17.5 kUA/L (classes 4–6).

The determination of sIgE has enabled the classification of allergens into two groups: (i) major allergens, which induce a positive skin prick test in more than 90% of allergic individuals, and (ii) minor allergens, which elicit a positive prick test in fewer than 20% of individuals allergic to the same allergen source (e.g. birch pollen or egg). Continuous exposure to low doses of allergens (e.g. house dust mites) induces greater sIgE synthesis than seasonal exposure to multiple pollen allergens.

Although the reduction in sIgE concentrations following allergen-specific immunotherapy (AIT) is not linear, sIgE represents an important indicator of immunological change (13,14). Due to immune system stimulation, sIgE concentrations transiently increase during the first 3–6 months of AIT (15). Between 6 and 12 months, concentrations stabilize and subsequently decrease as a result of Th2-response suppression, with a gradual replacement of IgE by IgG4 blocking antibodies. Two to three years after initiation of AIT, sIgE concentrations decrease by approximately 30–50%. With continued AIT, sIgE levels continue to decline slowly but never completely disappear. In most patients, a significant reduction in sIgE compared with baseline values is observed 2–5 years after the initiation of AIT (16). Therefore, sIgE can also be considered a pharmacodynamic biomarker.

Because values obtained by immunochemical methods depend on the analytical technique used, the same method (e.g. FEIA or CLIA) should be applied for longitudinal monitoring of sIgE concentrations. Nevertheless, better indicators of AIT efficacy include the measurement of IgG4 and the basophil activation test (BAT). Clinical improvement is usually observed within the first months of therapy, often earlier. Symptoms of allergic rhinoconjunctivitis improve faster (approximately two months) than asthma symptoms (6–12 months), and improvement occurs earlier in pollen and house dust mite allergy than in mold or animal dander allergy. The therapeutic effect typically persists for several years after discontinuation of AIT, after which sIgE concentrations may increase again (17).

Limitations of sIgE determination include the use of allergen extracts, which do not allow differentiation between genuine and cross-reactive allergens.

5. Component-Resolved Diagnostics

A significant advancement in laboratory diagnostics of allergies is component-resolved diagnostics (CRD), also referred to as molecular diagnostics, in which concentrations of specific IgE (sIgE) to individual allergenic molecules are determined. This approach enables the assessment of patterns of allergic sensitization at the molecular level (11, 18).

To date, a large number of natural and recombinant allergenic protein molecules have been isolated or produced for diagnostic use. Examples include molecules from birch (*Betula verrucosa*) (8 molecules, e.g. Bet v 1, Bet v 2, Bet v 4), timothy grass (*Phleum pratense*) (12 molecules, e.g. Phl p 1, Phl p 4), olive tree (*Olea europaea*) (15 molecules, e.g. Ole e 1, Ole e 7, Ole e 9), and house dust mite (*Dermatophagoides pteronyssinus*) (> 20 molecules, e.g. Der p 1, Der p 2, Der p 10).

4 Molekule prirodnih (*natural*, n) alergena dobivaju se pročišćavajnjem alergenskih ekstrakata kemijskim, kromatografskim, elektroforetskim i/ili imunoafinitetnim metodama. Tako se dobivaju npr. alergenske molekule proteina breze (Bet v1), ambrozije (nAmb a1), kikirikija (Ara h 8), mlijeka (nBos d 4, nBos d 5, nBos d 6, nBos d 8), itd.

5 Rekombinantne alergenske komponente proizvode se metodama genetskog inženjerstva. Služe za dobivanje npr. rekombinantnih (r) alergenskih molekula, npr breze (rBet v1, rBet v2, rBet v4, itd.), ose (rVes v 1, rVes v 3, rVes v 5), lateksa (rHev b 5) pčelinjeg otrova (rApi m1, rApi m2, rApi m10).

According to their chemical structure, allergenic molecules are proteins or peptides and belong to different protein families (19), some of which are presented in Table 1.

Allergenic molecules may be classified as genuine (primary) or cross-reactive. Cross-reactivity is defined as the ability of sIgE, in addition to binding genuine allergenic molecules, to bind other homologous allergenic molecules. While genuine allergenic molecules in atopic individuals trigger clinically significant allergic symptoms (e.g. anaphylaxis, angioedema, urticaria), cross-reactive molecules usually induce a weaker immune response and milder clinical manifestations, such as oral allergy syndrome (OAS).

OAS occurs as a result of cross-reactivity between inhalant allergenic molecules from tree, weed, and grass pollens and food allergenic molecules present in raw fruits and vegetables. For example, individuals sensitized to Amb a 8 and Bet v 2 may exhibit cross-reactivity with allergenic molecules from watermelon, melon, cucumber, and pumpkin (20). Similarly, the birch allergen Bet v 1—primarily responsible for respiratory allergy—can cause OAS due to cross-reactivity with allergenic molecules from raw apple, cherry, pear, and peanut (Mal d 1, Pru ar 1, Pyr c 1, Ara h 8). Thermal processing and acidification of raw fruits and vegetables (e.g. fresh thick smoothies) prevent the occurrence of OAS.

Table 1. Classification of selected allergenic molecules into different protein families*

Protein family	Inhalant allergens	Food allergens
Seed storage proteins	Hazel: Cor a 9, Cor a 11, Cor a 14	Peanut: Ara h 1, Ara h 2, Ara h 3, Ara h 4, Ara h 6, Ara h 7; Walnut: Jug r 1, Jug r 2, Jug r 6
Non-specific lipid transfer proteins (nsLTPs)	Ragweed: Amb a 6; Mugwort: Art v 3; Olive: Ole e 7; Pellitory: Par j 1	Peach: Pru p 3; Peanut: Ara h 9; Apple: Mal d 3; Walnut: Jug r 3
PR-10 proteins	Birch: Bet v 1; Hazel: Cor a 1; Oak: Que a 1	Peanut: Ara h 8; Apple: Mal d 1; Apricot: Pru ar 1; Carrot: Dau c 1; Soybean: Gly m 4
Profilins	Birch: Bet v 2; Hazel: Cor a 2; Oak: Que a 2; Timothy grass: Phl p 1; Ragweed: Amb a 8; Mugwort: Art v 4	Apple: Mal d 4; Peach: Pru p 4; Peanut: Ara h 5
Lipid-binding proteins	House dust mite: Der p 2, Der p 13; Ragweed: Amb a 6	–
Lipocalins	Cattle: Bos d 2; Dog: Can f 1, Can f 2, Can f 4, Can f 6; Cat: Fel d 4, Fel d 7	Beef: Bos d 5, Bos d 6
Polcalcins	Timothy grass: Phl p 7, Cyn d 7; Ragweed: Amb a 9, Amb a 10; Mugwort: Art v 5; Birch: Bet v 3, Bet v 4; Olive: Ole e 3, Ole e 8	–
Enzymes	Ragweed: Amb a 1, Amb a 2, Amb a 11, Amb a 12; Birch: Bet v 6, Bet v 7	–
Defensin-like proteins	Ragweed: Amb a 4; Mugwort: Art v 1	–
Serum albumins	Dog: Can f 3; Cat: Fel d 2; Chicken: Gal d 5	–
Tropomyosins	House dust mite: Der p 10, Der f 10	–

* Knowledge of the protein family to which individual allergenic molecules belong is crucial, as the type of allergenic molecule—and consequently its allergenicity—determines the severity of allergic symptoms. Hypersensitivity caused by stable proteins (such as seed storage proteins and non-specific lipid transfer proteins) is associated with a higher risk of severe allergic reactions. In contrast, when hypersensitivity is caused by labile proteins (e.g. pathogenesis-related protein 10 [PR-10] and profilins), patients typically experience milder, localized symptoms.

In clinical practice, two forms of component-resolved diagnostics are used: singleplex and multiplex methods. The singleplex method determines sIgE concentrations to individual allergenic molecules, whereas the multiparametric multiplex method simultaneously measures sIgE concentrations to a large number of allergenic components. Multiparametric diagnostic tests such as ImmunoCAP Immuno-Solid phase Allergen Chip (ISAC™), Allergy Explorer (ALEX™), and EUROLINE allow simultaneous detection of numerous allergen-specific IgE antibodies. The ISAC™ FEIA-based method detects approximately 112 components; ALEX2™ measures more than 170 parameters, combining extracts and components and detecting cross-reactive carbohydrate determinants (CCD); and the EUROLINE CLIA-based method determines specific panels combining extracts and individual components (20–22).

Results obtained by the ISAC method are expressed in semi-quantitative ISAC Standardized Units (ISU) across four categories: (i) < 0.3 ISU (undetectable sIgE; no sensitization); (ii) 0.3–0.9 ISU (low sIgE concentration); (iii) 1.0–1.9 ISU (moderately elevated sIgE concentration); and (iv) ≥ 15 ISU (significantly elevated sIgE concentration) (20). Results of the ALEX2™ method are expressed quantitatively in kUA/L across five classes, while EUROLINE results are expressed semi-quantitatively in arbitrary units across five classes. The ISAC method (FEIA) is considered the gold standard for CRD.

The advantages of CRD lie in its ability to identify sensitization to individual allergenic molecules, distinguish genuine from cross-reactive molecules, and consequently predict disease severity. It also aids in assessing the risk of anaphylaxis, for example in peanut allergy, where strong reactions to Ara h 2 contrast with mild reactions to Ara h 8 (23). In addition, CRD is useful in diagnostically unclear cases and in polysensitized patients. Concentrations of sIgE to individual allergenic molecules may therefore be regarded as diagnostic, prognostic, and predictive biomarkers.

Multiparametric testing simultaneously provides: (i) a broad allergen profile comparable to conventional sIgE testing using allergen extracts; (ii) a detailed profile of individual allergenic molecules (genuine and cross-reactive); and (iii) negative sIgE results for a wide range of non-allergenic molecules. In this way, laboratory allergy diagnostics increasingly align with personalized and precision medicine (24).

The rational use of CRD can be highly beneficial for patients when comprehensive results are interpreted correctly. Otherwise, it may represent unnecessary use of costly diagnostic tests. Limitations of CRD include complex interpretation requiring appropriate expertise, lower sensitivity of multiplex tests compared with singleplex assays, and high cost (approximately €250–300), which limits broad accessibility. Although various CRD platforms are available in routine practice, preference should be given to methods based on the more sensitive FEIA[4] technique rather than spectrophotometric (colorimetric) methods[5] (24).

6. Basophil Activation Test

The basophil activation test is a functional assay that assesses whether a tested allergen, after binding to IgE on basophils, is capable of inducing basophil activation and degranulation (24). CD203c is considered a marker of basophil activation[6], while CD63, released from lysosomes, is regarded as a marker of degranulation.

For stimulation, allergen extracts as well as individual allergenic molecules from various

4 ImmunoCAP ISAC

5 ALEX, EUROLINE

6 Klaster diferencijacije, CD (engl. *claster of diferentiation*)

sources may be used, including drugs (e.g. cephalosporins, H_2 blockers, and muscle relaxants), Hymenoptera venoms, and foods (e.g. peanut) (25). BAT shows higher specificity when stimulation is performed with individual allergenic components (4). The introduction of flow cytometry has significantly improved *ex vivo* cellular diagnostics; consequently, CD203c and CD63 expression on basophils is routinely assessed by flow cytometry following stimulation.

Interpretation of BAT results is based on the percentage of basophils expressing CD63 and/or CD203c after stimulation with a specific allergen. Reference values depend on the type of allergen and are not internationally standardized. The cut-off value for CD63-positive basophils may vary depending on the allergen and typically ranges from 4% to 15%. The assay includes a negative control (phosphate-buffered saline) and a positive control (anti-FcεRI antibodies), and a stimulation index (ratio of allergen-induced activation to negative control activation) is calculated; a value ≥ 2 is considered positive. BAT has diagnostic, predictive, and pharmacodynamic relevance (25).

The main advantage of BAT is that this quantitative test measures functional basophil activation *in vitro* and demonstrates high diagnostic specificity (with variable sensitivity) for clinically relevant allergens. The test is safe for patients, as it does not require in vivo allergen exposure. BAT enables assessment of the risk of anaphylaxis associated with drug administration, Hymenoptera stings, or exposure to certain allergens.

Corticosteroids, anti-IgE antibodies, and immunosuppressive therapy should be discontinued at least one week prior to blood sampling for BAT. A major limitation of BAT is the requirement for fresh blood samples; blood should ideally be processed within 4–24 hours after collection. Delayed processing (e.g. after 24 hours) may result in false-negative results (26,27).

For reliable results, the timing of blood sampling in relation to symptom onset is crucial. During an acute allergic reaction, basophils may already be degranulated, leading to false-negative BAT results. Approximately seven days after symptom onset, functional basophils are again present in circulation, allowing BAT to be performed. In patients with antibiotic-induced anaphylaxis, BAT should be conducted several weeks after antibiotic exposure, ideally three to four weeks later, to ensure reliable results (28).

7. Tryptase

β-tryptase is a protease released from the secretory granules of mast cells. Serum or plasma tryptase levels increase within 30–60 minutes after the onset of an anaphylactic episode and typically return to reference values within 24 hours (29). When anaphylaxis is suspected, blood sampling should be performed at three time points: (i) within 15–180 minutes during the acute event; (ii) 3-6 hours after symptom onset to detect possible complications; and (iii) 1–2 weeks after the anaphylactic episode, when tryptase concentrations are expected to be within the normal range. Measurement of tryptase activity has diagnostic and prognostic value.

Tryptase is also useful in provocation tests as an additional objective criterion, particularly in drug allergy. This elective setting allows comparison of tryptase levels with baseline values. Tryptase concentration is measured before provocation and after each incremental increase in the provocation dose. A clinically significant increase is defined as a concentration exceeding 1.2 × baseline tryptase (μg/L) + 2. For example, if the baseline tryptase concentration is 7.0 μg/L, a significant increase would be 1.2 × 7.0 + 2 = 10.4 μg/L (30). Such an increase represents a relevant finding indicating mast cell degranulation, even though reference values vary among laboratories (e.g. < 11.0 or < 13.5 μg/L). Approximately 95% of healthy individuals have tryptase concentrations below 13.5 μg/L, and about 90% have levels below 9.8 μg/L.

Table 2. Scope and limitations of parameters used in laboratory allergy diagnostics

Method	Parameter measured	Biomarker type	Advantages	Limitations
Eosinophil count	Number of eosinophils	Prognostic	Simple, widely available, inexpensive, no additional equipment required	Low specificity; does not reflect activation or degranulation; high intraindividual variability
Eosinophil cationic protein (ECP)	Activation, degranulation, and functional activity of eosinophils	Prognostic, pharmacodynamic	Reflects eosinophil activation rather than cell count; correlates with airway inflammation; useful for therapy monitoring	Preanalytical interferences: clinical (intraindividual variability, parasitic and viral infections, corticosteroid therapy, initiation of allergen-specific immunotherapy) and analytical (sample temperature and clotting time, serum vs plasma, hemolysis, lipemia)
Total IgE	Free IgE in serum	Diagnostic	Standardized according to World Health Organization reference standard	Low specificity; may be normal in allergic individuals or elevated in non-allergic individuals; method-dependent variability; marked intra- and interindividual variability; cannot replace skin testing or sIgE determination
Specific IgE	IgE to individual allergens; identification of sensitization	Diagnostic, pharmacodynamic	Identifies sensitization to allergen extracts; enables monitoring of immunotherapy	Does not distinguish sensitization from clinical allergy; results depend on analytical method
Component-resolved diagnostics	IgE to individual allergenic molecules; prediction of reaction severity; differentiation between genuine and cross-reactive molecules	Diagnostic, prognostic, predictive	Precise molecular allergen profiling; identification of high-risk proteins; improved immunotherapy selection; prediction of disease severity and anaphylaxis risk	High cost; complex interpretation requiring expertise; lower sensitivity of multiplex compared with singleplex assays
Basophil activation test (BAT)	Basophil activation and degranulation in the presence of allergens ex vivo (especially drugs and foods); useful when sIgE is negative	Diagnostic, predictive	Measures functional basophil activation in vitro; high specificity for clinically relevant allergens	Complex ex vivo method; requires expensive equipment and fresh blood; limited availability; lack of full standardization for most allergens
Tryptase	Mast cell degranulation	Diagnostic, prognostic	Rapid kinetics (30–120 min after allergic reaction) support confirmation of acute anaphylaxis; helps differentiate IgE-mediated anaphylaxis from other shock or urticaria episodes	Not specific for allergy (elevated in mastocytosis); strong dependence on timing of blood sampling (1–4 h after reaction); delayed sampling may lead to false-negative results

8. Future of Laboratory Diagnostics in Allergy

The foundation of advanced artificial intelligence lies in information technology, which integrates computer systems (hardware, software, and peripheral equipment), programming languages, data processing, and data storage, and requires appropriately trained information technology specialists (31).

In the future, procedures for the simultaneous detection of multiple allergenic molecules will be further improved and automated, including BAT multiplex. These advancements will shorten the time required to obtain standardized and reproducible BAT results, enable clearer discrimination between positive and negative responses, optimize activation threshold cut-off values for basophils, and allow bioinformatic algorithms to identify dominant allergens within multiplex panels. Collectively, these developments will facilitate broader clinical application of BAT and further advance personalized allergy medicine (32).

8.1. Omics Technologies

In the future, omics technologies will provide a comprehensive insight into the molecular mechanisms underlying allergic diseases. These include genomics (monogenic and polygenic disease foundations, genetic predisposition, and therapeutic response), epigenomics (the influence of environmental factors on gene regulation), proteomics (gene expression profiles, inflammatory biomarkers, structural modifications of allergens, and interactions between proteins and IgE), transcriptomics (expression of inflammatory and immune-regulatory genes across different patient phenotypes and disease endotypes[7]), and metabolomics (profiling of small molecules involved in inflammation and oxidative stress) (33).

Advances in these fields will enable the discovery and validation of novel biomarkers that define characteristic patterns of allergic inflammation endotypes. Such patterns will contribute to predicting treatment response, enable personalized therapeutic approaches, and support the prediction of therapeutic outcomes. An integrated multi-omics approach requires advanced bioinformatic analysis and ultimately leads to successful personalized medicine and the development of new therapeutic strategies (34,35).

8.2. Advanced Artificial Intelligence

Advanced artificial intelligence (AI) will be gradually integrated into laboratory allergy diagnostics following the harmonization, validation, and standardization of individual algorithms. These algorithms will be developed based on the combined expertise of specialists in laboratory and clinical medicine, information technology professionals, and evidence-based medicine principles (36). A key role in the integration of advanced AI algorithms will be played by information technology experts, including software developers responsible for designing and maintaining the required systems.

By integrating AI models—including machine learning, deep learning, neural networks, and clustering techniques—with large datasets generated through laboratory allergy diagnostics (both existing and emerging methods), clinical laboratories will evolve into “smart laboratories.” Such laboratories will be capable of sophisticated processing of relevant *in vitro* analytical results and *ex vivo* test data, as well as identifying co-sensitization, polysensitization, and molecular-level cross-reactivity.

When data from laboratory information systems are integrated with clinical data (e.g. symptoms, skin test results, pulmonary function tests) for individual patients, allergists will be able

7 A phenotype refers to the observable characteristics of an organism as they develop under the influence of environmental factors in interaction with the genotype (inherited traits). An endotype is a subtype of a health condition defined by a distinct functional or pathobiological mechanism. It is characterized on the basis of a specific pathophysiological mechanism rather than solely by clinical symptoms.

to more accurately predict the development and outcome of allergic diseases, select and apply AIT, and anticipate therapeutic responses. In other words, this integration will enable personalized allergy diagnostics and personalized pharmacotherapy (37,38). Supported by medical expertise, a competent allergist will be able to overcome the limitations of advanced AI systems.

Ultimately, patients will benefit substantially, including faster and more accurate diagnostics, personalized treatment strategies, and support through mobile applications—such as reminders for prescribed medication intake and potential prediction of disease exacerbations requiring allergist intervention.

The integration of existing and emerging laboratory allergy diagnostic methods—including omics technologies—with artificial intelligence marks the beginning of a new and challenging era in medicine, characterized by improved diagnostic accuracy, personalized approaches, and a deeper understanding of complex and yet undiscovered pathomechanisms. At the same time, this progress raises important ethical issues (data privacy) and legal considerations (regulatory compliance) that must be carefully addressed (39).

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