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Basophil activation test (BAT): Clinical and research relevance in allergy diagnostics

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ABSTRACT

The basophil activation test (BAT) is transitioning from a research assay to a clinically actionable tool. By capturing IgE-mediated basophil degranulation in vitro, BAT can complement history, skin testing, and serum specific IgE. High-quality studies in food allergy show the strongest evidence for peanut and sesame, where BAT improves diagnostic accuracy and may obviate oral food challenges. In drug allergy, BAT's high specificity can confirm culprit sensitization in defined contexts and, uniquely, screen potential alternative agents when drug provocation is hazardous. Additional applications include confirming clinically relevant sensitization prior to respiratory allergen immunotherapy, refining diagnosis and risk stratification in Hymenoptera venom allergy, and endotyping in chronic urticaria.

Implementation has been limited by logistics, the need for experienced flow-cytometry staff, and historical heterogeneity in markers, panels, and positivity thresholds. Crucially, the EAACI published an externally quality-assured protocol with a standard operating procedure. This standardization establishes a foundation for controlled multicenter research.

Importantly, in selected scenarios – such as some equivocal IgE-mediated food allergies and severe immediate drug reactions where provocation test may be unsafe – BAT can help reduce or avoid the need for provocation testing when available and appropriately interpreted. Priority next steps are broad protocol adoption and prospective, multicenter validation to define robust, setting-specific decision thresholds and patient-relevant outcomes.

KEY WORDS: Basophil Activation Test (BAT), allergy diagnostics, food allergy, drug allergy, personalized medicine

INTRODUCTION

The current gold standard in allergy diagnostics remains the allergen provocation test. However, this procedure is time-consuming, resource-dependent, and carries a risk of severe reactions.

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Consequently, alternative approaches have been explored to reproduce effector-cell responses to allergens without exposing patients to unnecessary risk. Because immediate hypersensitivity reactions are mediated by basophils in response to allergen cross-linking of surface immunoglobulins E (IgE), the basophil activation test (BAT) has been developed to model this biology in vitro. In vitro cell-activation assays are widely used in clinical research and, increasingly, in routine practice; however, availability is heterogeneous across centers. Until recently, the lack of consensus on technique standardization and inter-laboratory reproducibility limited routine use; however, an EAACI Task Force has now provided a consensus, externally quality-assured BAT protocol. As this newly standardized approach is adopted, it will take time for centers to adjust and for research to provide consistently comparable results across studies [1]. This review summarizes recent findings on the application of BAT in food, respiratory, Hymenoptera, and drug allergy diagnostics.

BAT TECHNIQUE

The BAT is a flow-cytometric assay that indirectly reflects histamine release by quantifying activation markers on the basophil membrane [2,3]. BAT closely approximates the in vivo response by assessing activation of live basophils following allergen-induced IgE cross-linking. Activation, in other words, measures degranulation with histamine release by flow cytometry. We next outline the procedure step by step.

Workflow

Fresh whole blood is collected [2,3]. The test allergen is added at multiple concentrations to separate tubes and incubated for protocol-specified durations. Positive and negative control tubes undergo identical handling, with the negative control lacking allergen. A "master mix" of antibodies for basophil identification and degranulation is then added and incubated. Red cells are lysed, and samples are prepared for acquisition. This process is summarized in Figure 1.

Gating/identification

Basophils are identified using laboratory-specific panels [2,3]. Degranulation is assessed by expression of CD63 or CD203c on basophils via flow cytometry.

Markers

CD63 is currently the most used readout for basophil activation. Resting basophils lack surface CD63; the marker resides on secretory lysosomes[2]. Upon activation, histamine-containing granules fuse with the plasma membrane, externalizing CD63, the expression of which correlates closely with histamine release [2]. Some laboratories use CD203c as an alternative or complementary marke r[2,3].

Controls and non-responders

Two positive controls should be included [2,3]. For a non-IgE-mediated stimulus, many laboratories use the bacterial tripeptide fMLP, which activates basophils via G-protein–coupled pathways independently of the IgE/FcεRI complex [2,3]. For an IgE-mediated positive control, anti-IgE or anti-FcεRI is used [2]. In up to 10% of tests, basophils fail to respond to IgE-mediated stimulation; these samples are labeled as "non-responders" [2]. For the negative control, PBS or an equivalent buffer is used.

Readouts and performance characteristics

When CD63 is used, results are typically expressed as the percentage of CD63c basophils. When CD203c is adopted, the results are presented as mean fluorescence intensity (MFI) fold-change relative to the negative (PBS) control [3]. In most cases, results are dose-dependent, showing a higher proportion of activated basophils at increasing allergen concentrations. Dose–response curves

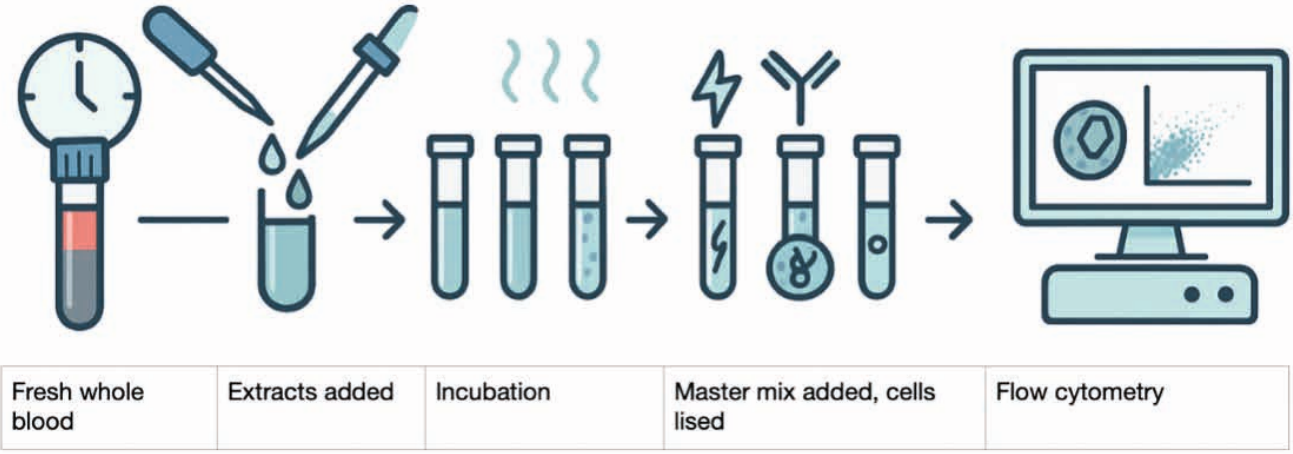


FIGURE 1. BAT workflow.

vary across individuals and even across allergens within the same individual [3,4]. There is no universal cutoff that defines a positive BAT; thresholds depend on the allergen tested [5].
Basophil sensitivity refers to the allergen dose that causes activation in 50% of basophils, also expressed as CD-sens [3]. The considerations for BAT technique are summarized in Table I.

Standardization and EQA

In 2024 the EAACI BAT-EQA Task Force made available a standard operating protocol together with reference materials to standardize and enhance test accuracy between centers. The protocol can be implemented across Europe, including workflows with pre-activated blood [1].

BAT IN FOOD ALLERGY

The use of the BAT in food allergy has been extensively investigated, including evidence from meta-analyses. International evidence-based guidelines have recently introduced BAT in clinical practice in specific scenarios. EAACI guidelines, in particular in patients with equivocal diagnosis of IgE-mediated allergy to peanut or sesame, state that when BAT is available there is high recommendation to perform it to confirm the diagnosis due to its high sensitivity and specificity [4,8]. Several studies also indicate that BAT can differentiate between sensitization and clinical allergy to egg and milk [4].

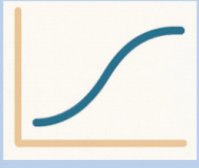
BAT in peanut allergy

BAT has been most extensively studied in peanut allergy. Its high sensitivity and specificity make it a valuable diagnostic tool. A positive result may obviate the need for a risky oral food challenge (OFC), whereas a negative BAT can support proceeding with OFC in sensitized children, thereby preventing unnecessary dietary restrictions [2,9]. Across multiple studies, higher basophil reactivity to peanut has been associated with greater reaction severity [2].
BAT has also been applied to monitoring oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) [5,10,11]. In combined omalizumab and peanut OIT, BAT demonstrated a reduction in basophil allergen threshold sensitivity (CD-sens) during omalizumab pre-treatment, and a further decrease throughout OIT [5]. Both basophil reactivity and CD-sens increased when omalizumab was reduced, with higher values observed in treatment failures [5]. CD-sens has also been used to guide omalizumab dose escalation during OIT [12]. Moreover, BAT has been reported to predict sustained unresponsiveness in patients undergoing peanut OIT [11,13].
In a study on 74 patients, indirect BAT using Ara h 2 and Ara h 6 showed excellent accuracy (94%). Indirect BAT uses donor basophils passively sensitized with patient IgE, allowing sample processing that is not restricted to the day of blood collection [14].

BAT in egg allergy

Growing evidence supports the utility of BAT in hen's egg allergy for risk stratification, diagnosis, and treatment monitoring. In a study by Radulovic et al., BAT outperformed ovomucoid specific IgE in predicting both severity and threshold for baked-egg reactions [15]. A reduction in CD63 expression after egg immunotherapy has also been reported [9].
Another study by Licaeri et al. showed that BAT to egg white/yolk

TABLE I. Considerations of the BAT technique.

Pre-Analytical	Analytical Setup	Flow-Cytometry	Interpretation
			
• Blood should be processed as soon as possible. Testing within 24 h is recommended [2]. • Basophil reactivity is sensitive to vibration, temperature changes, and the anticoagulant used [6]. • Concomitant infection alters basophil responsiveness and is a contraindication for BAT [7]. • Systemic glucocorticoids suppress activation and should be stopped ≥3 weeks before testing [7].	• Use standardized allergen extracts or parenteral drug formulations [2]. • Test in increasing concentrations. Dose producing positive response varies by patient and allergen [2]. • If basophils fail to respond to anti-IgE and allergens, the assay is inconclusive [3].	• Basophil identification: No unique marker; require ≥2 markers (e.g., CCR3 + CD123 or CD203c + HLA-DR-) [3]. • Adequate basophil count required for valid result [3]. • Flow cytometry is quasi-quantitative. Absolute accuracy limited [3]. • Instrument/software differences can shift readouts by ~10% [7].	• No universally accepted cut-offs for assay positivity [3]. • Cross-study comparisons are difficult due to heterogeneity in protocols, reagents, and data analysis [7].

discriminates sensitization from true allergy. Patients with a positive OFC had significantly higher CD63 expression (e.g., 44.4% vs 12.5% for egg white) [16].

BAT can also be configured using progressively more cooked forms of egg. De Vlieger et al. reported 86.1% concordance between BAT and OFC, with 13.9% false negatives and an unexpectedly high proportion (20%) of non-responsive basophils [17].

BAT in milk allergy

In a study involving 150 children with suspected milk allergy who underwent open food challenge, BAT demonstrated the highest diagnostic accuracy in comparison with specific IgE and skin prick test. Notably, in a subgroup of children under 2 years of age, applying BAT as a triage test meant that only 27% would have needed an OFC, thereby potentially reducing exposure to challenge-related risk [18].

BAT in other food allergies

Additionally, there is emerging data on the use of BAT in allergy to tree nuts, sesame, fish, and LTP, showing high specificity and providing information on cross-reactivity [19-23].

BAT IN HYMENOPTERA VENOM ALLERGY

Hymenoptera venom allergy (HVA) is a global problem and a major cause of anaphylaxis, sometimes fatal. Many patients cannot identify the culprit insect, and in Polistes and vespid wasps there is marked cross-reactivity, making component diagnostics insufficient to confirm the species. When venom immunotherapy is indicated, EAACI explicitly includes the basophil activation test (BAT) among acceptable diagnostic tests and further gives a weak recommendation against using BAT to estimate individual risk of relapse [24].

Diagnostic utility

The BAT can confirm HVA when skin tests are negative or sIgE is undetectable [2,7]. Moreover, BAT helps differentiate true double sensitization from cross-reactivity in HVA [2,25]. In a study by Cabrera et al., CAP-inhibition (reference method) showed 59.46% sensitivity with 15/37 indeterminate results. In a subset, BAT showed higher sensitivity than CAP-inhibition ($p = 0.021$), 71.43% positive agreement, identified 100% of inconclusive cases, and demonstrated 83.3% specificity [26]. It has also been suggested that BAT results can predict adverse reactions during venom immunotherapy and its efficacy in both children and adults [27-29]. In a study with 34 patients, those who developed systemic reaction to venom immunotherapy had a significantly higher percentage of activated basophils after stimulation with culprit venom [30]. This information could be used to perform slower up-dosing of immunotherapy in selected patients to offer a safer approach.

BAT IN DRUG ALLERGY

BAT is gradually being integrated into drug-allergy work-ups. It has been shown to have high specificity for several medications, allowing omission of drug challenges when BAT is positive [31]. Additionally, BAT permits assessment of the culprit drug and potential alternatives in parallel. It is also of benefit if there is a contraindication for drug challenge like life-threatening anaphylaxis [32].

According to recent EAACI guidance on BAT in drug allergy, there is a strong recommendation to use BAT, when available, as an initial step following life-threatening anaphylaxis to drugs. EAACI also strongly recommends BAT for the diagnosis of immediate hypersensitivity to β-lactams, neuromuscular-blocking agents (NMBAs), and chlorhexidine [32]. Conversely, EAACI issues a strong recommendation against the use of BAT for the evaluation of non-allergic immediate reactions to NSAIDs and for suspected reactions to COVID-19 vaccines [32].

Antibiotics

Overall, BAT revealed high specificity (92%) but lower sensitivity for antibiotics (40%) [31]. In 18 patients with confirmed cefazolin anaphylaxis, BAT demonstrated 38-75% sensitivity, respectively [33]. In contrast, sensitivity has been low in amoxicillin allergy, 13% with CD63 and 23% with CD203c in a study including 66 allergic patients and 70 controls, limiting its utility as an alternative to drug provocation in that context [34]. For fluoroquinolones, published evidence remains conflicting and no consensus has been reached. The EAACI position paper provides summary tables by drug group to facilitate implementation in routine practice [32].

Non-steroidal anti-inflammatory drugs (NSAIDs)

In non–IgE-mediated NSAID hypersensitivity, BAT performance has been variable, with individual studies reporting sensitivities of 37-56% and specificities of 75-90% for aspirin [35,36].

Neuromuscular blocking agents (NMBAs)

For NMBAs, BAT is consistently reported to have high specificity, with sensitivity spanning approximately 36-100% across studies. BAT may be particularly informative in the setting of negative skin tests [32].

Intravenous contrast media

Data for intravenous contrast media are more limited. In a small study with gadolinium-allergic, BAT was positive in at least one case with negative skin testing, suggesting potential incremental diagnostic value [37].

Chemotherapeutics

Chemotherapeutic agents present a distinct set of challenges. Hypersensitivity reactions are common, skin tests are frequently negative, often reflecting non-IgE mechanisms, and cytotoxicity imposes procedural constraints on laboratory handling [38].

Nevertheless, emerging evidence suggests its utility: in a study by La Sorda et al., most paclitaxel reactors were skin-test negative but BAT-positive, consistent with possible MRGPRX2-mediated activation, whereas taxane reactors were commonly positive on both skin testing and BAT. The combined information from skin tests and BAT could be used to plan the desensitization process and its monitoring, with BAT correctly identifying 78.57% of paclitaxel- and 90.91% of taxane-hypersensitive patients [39]. In a larger series of 121 patients with suspected chemotherapy allergy, BAT demonstrated 79% sensitivity for IgE-mediated reactions to platinum salts and correlated well with skin testing [40]. Overall, however, BAT applications in oncology remain under-investigated and limited by safety and logistical considerations [38].

BAT OF DIAGNOSTIC UTILITY

BAT in respiratory allergy

Current EAACI guidelines [41,42] on respiratory allergy and asthma do not mention BAT for routine diagnostic use, indicating that its clinical application is currently limited to research settings and highly specialized centers.

The BAT may have a useful role in respiratory allergy, especially to support diagnosis before initiating allergen immunotherapy, a treatment that is both costly and long-term. In a study by Spiewak et al. on house-dust-mite (HDM)–allergic children with controls allergic to other inhalants, BAT showed 90.6% sensitivity and 100% specificity, and confirmed high cross-reactivity between *D. pteronyssinus* and *D. farinae* [43]. Other authors have likewise reported high sensitivity and specificity of BAT in HDM and pollen allergy [44].

A potential practical advantage is the ability to test the specific extract from the manufacturer intended for immunotherapy, which may support personalized extract selection. However, this should be viewed as supportive rather than deterministic evidence of immunotherapy success and does not replace standard clinical criteria.

Beyond diagnosis, BAT-derived basophil allergen threshold sensitivity (CD-sens) may reflect lower-airway biology. In a study of 26 asthmatic patients, CD-sens correlated with airway allergen sensitivity both for allergen PD20 and the allergen-to-methacholine PD20 ratio. Therefore, CD-sens could serve as an objective marker of clinically relevant IgE sensitization and help anticipate airway responsiveness when bronchial challenge testing is not feasible [45].

BAT in urticaria

In chronic urticaria, the BAT may help to delineate clinically meaningful endotypes. The international EAACI/GA^{2}LEN/EuroGuiDerm/APAAACI mentions BAT as a possibility as a part of advanced testing in spontaneous urticaria to determine autoimmune subtype[46]. In a large cohort study by Pesqué et al., patients with a positive BAT more frequently exhibited low total IgE, as well as higher rates of anti–thyroid peroxidase IgG and autologous serum skin test (ASST) positivity [47]. Additionally, BAT-positive patients had higher omalizumab failure rates, suggesting this endotype may be better suited to alternative biologics when available [47].

BAT may also complement the ASST in supporting a diagnosis of autoimmune chronic urticaria. In 139 adults with chronic urticaria, concordant ASST and BAT positivity was associated with higher Urticaria Activity Scores and may aid risk stratification [48].

DISCUSSION

BAT is steadily transitioning from a research tool to a clinically actionable assay across multiple allergy domains, supported by an expanding body of high-quality studies. In food allergy, robust evidence, particularly for peanut and sesame, shows that BAT can increase diagnostic accuracy, reduce unnecessary oral food challenges, and monitor immunotherapy responses. In drug allergy, BAT's high specificity in selected settings can obviate drug provocation when positive and, crucially, allows parallel testing of culprit and potential alternative agents when challenges are contraindicated after severe reactions. Additional applications in respiratory allergy (pre-immunotherapy confirmation), Hymenoptera venom allergy (diagnosis, risk stratification, and venom immunotherapy guidance), and chronic urticaria (endotyping and prognostication) further demonstrate its clinical utility (Tab. II).

A central barrier to widespread adoption has been methodological heterogeneity. Differences in basophil identification panels, activation markers, stimulus preparation and concentration ranges, gating strategies, and positivity thresholds historically limited inter-laboratory comparability and day-to-day implementation. Importantly, a recent EAACI Task Force report provides a path forward: a consensus protocol with acceptable inter- and intra-laboratory variability by accepted standards, together with a standard operating protocol and reference materials. The protocol can be implemented across Europe, including workflows using preactivated blood, thereby laying the groundwork for controlled multicenter studies and routine EQA participation [1]. Despite this progress, practical constraints remain. BAT depends on fresh samples and skilled flow-cytometry personnel, and the problem of patients with non-responsive basophils remains. Continued prospective, multi-center work, especially in pediatrics and under-studied drug classes, is crucial to understand patient-important outcomes.

CONCLUSIONS

BAT is slowly but convincingly entering routine allergology for diagnosis and monitoring, backed by numerous high-quality investigations and selective endorsement in international guidance. Its principal clinical benefits include reducing or avoiding provocation challenges in food allergy and, in drug allergy, confirming culprit sensitization while identifying safer alternatives within the same assay session. The EAACI

TABLE II. Key notes for the clinical application of BAT in the main allergic diseases.

Allergy field	Main clinical applications	Guideline	Practical notes
Food allergy	• Distinguish sensitization vs clinical allergy • Refine diagnosis when IgE/SPT are equivocal • Monitoring treatment • Clarify cross-reactivity	• EAACI: recommends BAT (when available) to confirm equivocal IgE-mediated peanut or sesame allergy (currently no strong evidence for other foods)	• Positive BAT may avoid OFC • Negative BAT can support proceeding with OFC and prevent unnecessary dietary restrictions
Hymenoptera venom allergy	• Confirm HVA when skin tests/ sIgE are negative • Differentiate true double sensitization vs cross-reactivity • Risk stratification and VIT monitoring	• EAACI: BAT among acceptable diagnostic tests before VIT. • Weak recommendation against using BAT to estimate relapse risk	• Higher basophil activation associated with more severe systemic reactions during VIT, may justify slower up-dosing in selected patients
Drug allergy	• Diagnostic support when challenge is high-risk or contraindicated • Evaluate culprit and alternative drugs in parallel	• EAACI: strong recommendation after life-threatening anaphylaxis • strong recommendation against BAT in NSAID non-IgE reactions	• Specificity generally high, sensitivity drug-dependent • Most useful as part of an integrated work-up
Respiratory allergy / asthma	• Research tool to characterize clinically relevant sensitization and CD-sens (airway allergen sensitivity)	• Current EAACI respiratory/ asthma guidelines do not recommend or even mention BAT for routine use	• Not established for routine clinical decision-making
Chronic urticaria	• Define autoimmune endotypes • Risk stratification (higher disease activity, omalizumab non-response)	• International EAACI/GA^{2}LEN/ EuroGuiDerm/APAAACI urticaria guideline: BAT mentioned among advanced tests to identify autoimmune CSU	• BAT-positive patients more often show ASST positivity and higher omalizumab failure rates

ASST: autologous serum skin test; BAT: basophil activation test; CSU: chronic spontaneous urticaria; HVA: Hymenoptera venom allergy; NSAIDs: non-steroidal anti-inflammatory drugs; OFC: oral food challenge; SPT: skin prick test; VIT: venom immunotherapy.

Task Force's consensus protocol demonstrates that validation and standardization are achievable [1]. As these standards are adopted, BAT is poised to complement existing pathways, minimize patient risk, and enable more individualized therapy across the allergy spectrum.

Institutional Review Board Statement

Not applicable.

Conflicts of interest statement

With regards to the present work, the Authors have no conflict of interest to declare.

Authors' contributions

SA and MN conceived the manuscript, wrote the first draft, and finalized it. All authors revised the manuscript and approved the final version.

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