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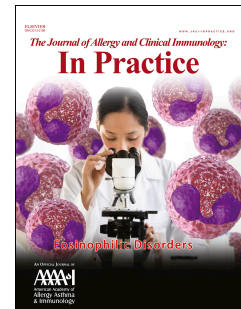
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Bringing the next generation of food allergy diagnostics into the clinic

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Abstract:

Food allergy diagnosis has a massive impact on the lives of patients and their families. Despite recent developments with specific IgE to component allergens, a significant proportion of

patients assessed for possible food allergy require oral food challenge (OFC) to ensure an accurate diagnosis. More precise diagnostic methods are required to reduce the need for OFCs. Bead-based epitope assays and cellular tests, such as basophil activation and mast cell tests (BAT and MAT, respectively), are the most novel and promising tests on the horizon. There is a pathway to pursue in order to enable their incorporation in clinical practice, including standardization, technical validation, clinical validation, external validation, overcoming practical and logistical issues, and regulatory approval. Valuable clinical application of these tests goes beyond diagnosis and includes risk assessment to identify the allergic patients who are most sensitive and at risk of severe allergic reactions and also to define prognosis and assess clinical response to immunomodulatory treatments.

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Keywords: Basophil activation test, Mast cell activation test, Epitope mapping, Food allergy, Diagnosis

Abbreviations:

BAT -basophil activation test

BBEA – bead-based epitope assay

DBPCFC – double-blind placebo-controlled food challenge

MAT – mast cell activation test

OFC – oral food challenge

RAST – radioallergosorbent test

sIgE – specific IgE

SPT – skin prick test

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Introduction

Diagnosing food allergy has major implications for daily life leading to dietary restrictions and constant risk assessment to minimise accidental exposure and allergic reactions, which can cause anxiety of varying severity and limit the activities that individuals can engage with, such as social events, eating at restaurants and travelling^{1, 2}. Over diagnosis can therefore cause unnecessary negative impact in patients' lives. Conversely, **failure to diagnose food allergy** can place the patient at risk of reacting to the food unexpectedly, with potential severe consequences. It is thus extremely important to get the diagnosis right.

Currently, food allergy is usually diagnosed based on a history of an acute allergic reaction that happens within 2 hours (usually minutes) of allergen exposure and evidence of allergen-specific IgE (sIgE) to the food in question. sIgE can be documented by skin prick test (SPT) or the measurement of sIgE in the serum using allergen extracts or individual components, which can be either natural purified or recombinant proteins. In spite of the improved accuracy conferred by sIgE to individual allergens for some foods, such as Ara h 2 from peanut and Cor a 9 and Cor a 14 from hazelnut, compared to IgE to allergen extracts, a significant proportion of patients being assessed for possible food allergy require oral food challenge (OFC), which is currently the gold-standard to diagnose food allergy. In fact, offering allergy tests, such as SPT and sIgE testing, should ideally come with access to OFC to minimise overdiagnosis of food allergy.

OFC is the current gold-standard for diagnosis, assessment of severity, identification of threshold dose of reactivity, confirmation of eligibility for treatment and assessment of response to treatment. However, OFCs have limitations, require intensive resources and can cause allergic reactions of varying severity. The need to undergo OFCs can leave patients and family unsettled and anxious, and may discourage allergic patients from considering specific treatments for their food allergy, if OFC are needed to confirm eligibility. Therefore, improved biomarkers for food allergy are needed to close these gaps, provide an accurate diagnosis and reduce the need for OFC.

The most novel and promising tests to support the diagnosis of food allergy in the pipeline, currently making the transition to clinical practice are: IgE to allergen peptides and cellular tests, such as the basophil activation test (BAT) and the mast cell activation test (MAT). We will focus on these tests in the next sections: following a description of these methods and their

clinical utility, we define a pathway to bring these new tests to the clinic and make them accessible to clinicians and patients with suspected food allergy.

The next generation of food allergy diagnostics

IgE to epitopes

Following the first *in vitro* assay capable of measuring sIgE in patient sera (radioallergosorbent test [RAST]) in a semi-quantitative way³, a quantitative assay, ImmunoCAP® (Pharmacia; Uppsala, Sweden), calibrated against the WHO standard IgE, provided improved sensitivity and predictive value in diagnosing clinical allergy.^{4,5} The quantity of IgE specific to milk, egg, peanut and fish were shown to correlate to the probability of reacting to the food when compared to the DBPCFC, and 95% predictive value cut-offs were suggested.⁶ Component proteins comprising various food allergens (e.g. milk casein, egg ovomucoid, peanut conglutins) eliminated many cross-reacting and clinically irrelevant allergen components and were more predictive of clinical reactivity than the whole food.⁷ Pursuing this reasoning further, it was postulated that more precise IgE profiling, i.e. evaluating epitope-specific antibodies found on allergenic component proteins, could improve diagnostic and prognostic methods by further eliminating irrelevant signals produced by cross-reactive and less specific antibodies. Early studies using SPOTS membrane technology suggested that IgE to sequential (linear) epitopes correlated most closely with persistent, systemic food-allergic reactions.⁸ Subsequent studies advanced to the use of microarray technology,⁹ and more recently to a high-throughput, highly reproducible bead-based epitope assay (BBEA).¹⁰

As seen in **Figure 1**, the BBEA is a multiplex assay capable of evaluating IgE and IgG4 to more than 90 peptide sequences representing various sequential epitopes. Advantages of the BBEA include the microliter quantities of serum or plasma required to perform the assay, its technical ease of performance and reproducibility, its greater degree of accuracy, and its potential to predict clinical outcomes. To date, most allergenic epitope profiling has focused on milk and peanut allergy (**Table 1**). While this technology appears to hold great promise, it requires knowledge of the amino acid sequences of component protein allergenic epitopes comprising each individual food, large cohorts of well-characterized food allergic patients to validate the assays, and extensive computational skills and machine learning to develop and validate diagnostic and prognostic algorithms for each food allergen.

Basophil and mast cell activation tests

More than the quantity of sIgE, the quality of sIgE is critical in determining the degree of mast cells and basophil activation, which are ultimately responsible for the allergic symptoms. Recently, Hemmings et al¹¹ have demonstrated that the proportion of IgE that is allergen-specific, the diversity of IgE response in terms of allergens that sIgE recognises, the avidity for binding to the allergen as well as sIgE levels, all contribute to the proportion of mast cells and basophils that become activated in response to allergen stimulation. The beauty of the cellular tests, like the BAT and the MAT, is that they take all the elements of sIgE into account when determining the likelihood that allergen exposure will lead to allergic reactions in an IgE sensitised individual¹². The BAT uses whole blood while the MAT uses plasma or serum to sensitize mast cells that can either be a cell line or primary cells derived from peripheral blood or from tissue. These basophils or mast cells are then stimulated with allergen and analysed by flow cytometry to detect the expression of activation markers before and after stimulation with allergens or controls (**Figure 2**)¹³.

Both allergen extracts and individual allergens can be used for cell stimulation, with different diagnostic performance, depending on the allergen source^{12, 14}. Generally, BAT has shown high sensitivity and specificity to diagnose peanut and other food allergies (**Table 1**)¹⁵⁻¹⁷. The MAT has shown similar specificity in the diagnosis of peanut allergy but lower sensitivity as this is a more artificial system requiring antibodies in a fixed volume of plasma to bind to receptors on the surface of mast cells and dependent on the density of such receptors on the cell surface¹⁸. As BAT and MAT require flow cytometry, it is not, with current methods, feasible to use them as a screening test but rather as a confirmatory test, as a second step in the diagnostic work-up in cases in which the first assessment with SPT or sIgE was not conclusive^{19, 20}. Used this way, the high specificity is the main added value to SPT and sIgE, which have high sensitivity but relatively lower specificity^{15, 16}. With more high-throughput methods, however, BAT, which also has a high sensitivity, could be used more widely. Although BAT has high accuracy, ca. 10-15% individuals with basophils that respond to non-IgE mediated stimulants but not to IgE-mediated control or allergen – the so-called non-responders, have an uninterpretable BAT¹³. In these cases, MAT which uses a passive sensitization system where only the plasma comes from the patient, can provide a conclusive result¹⁸.

Pathway to clinical practice

Different stages can be identified from the development of a putative diagnostic test to its clinical implementation (**Figure 3**); for instance: 1. standardization, 2. technical validation, 3. clinical validation, 4. external validation and 5. overcoming practical and logistical issues, towards 6. regulatory approval. We will address these in turn.

1. Standardization

Standardization of diagnostic tests allow for comparisons across time and laboratories and is therefore vital to their development for clinical utility. For instance, in the BBEA, length and sequence of peptides used on the beads, the manufacturer of the peptides, the type and size of beads and the equipment to measure IgE binding and data analyses which have included machine learning, are some of the aspects to consider. In the BAT and MAT assays, aspects that must be considered for standardization, include allergen sources, cellular markers for identification and activation, and data analysis.

Allergen contents of food extracts can vary widely depending on the source material and processing of the food (e.g. roasted vs raw peanut) as was recently demonstrated for peanut²¹. Source materials from extract providers with existing biological licenses, such as those used in SPT (e.g. Stallergenes/Greer and ALK), for instance provided in lyophilized form that can be readily resuspended are useful for BAT and MAT. Purified allergens, or combinations of allergens, can also be used but there are limited allergens available. However, there are companies offering some standardized allergens for purchase (e.g. Indoor Biotech; Charlottesville, VA). Another aspect of extract standardization is the concentrations of proteins to use in the assay. Typically, there is a log-fold dose range used in BAT assays ranging from 10 µg/mL to 1 ng/mL^{15, 17}. Since the BAT assay relies on peripheral blood, timing and transport of blood for testing must also be standardized. BAT assays can be performed within 24 hours of blood collection, however, methods, such as refrigeration of the blood²² or fixing the samples before acquisition²³, may allow to extend this timing.

BAT and MAT assays rely on flow cytometry to quantify activation and therefore choice of cellular markers to target with antibody staining are important. Basophils can be identified by a variety of cellular markers, including CD203c, FcεRI, IgE, CD123, CCR3, and mast cells by c-kit and FcεRI. Activation of basophils and mast cells is typically achieved by quantifying CD63 on the cellular surface¹³. Basophil activation can also be measured by change in fluorescence of CD203c with allergen stimulation¹³. Standardization of markers and antibodies to use for

identification and activation of these effector cells is critical and should balance complexity of the flow cytometric acquisition of the data with rigor in identifying the correct cell population. Flow cytometry is now routinely used in clinical laboratory settings and should be readily applicable for BAT and MAT assays. Data analysis also needs to be standardized and ideally this will move from manual gating strategies to more automated computer-assisted analysis^{24, 25}.

2. Technical validation

Technical validation of BBEA, BAT and MAT assays is important for transitioning these assays from research laboratories into clinical practice. For example, demonstrating the reproducibility of cellular activation on blood drawn from the same subject on the same day by several operators will need to be performed for the BAT assay in question. Additionally, data generated from blood processed immediately after collection will need to be compared with blood processed several hours, or possibly days later to determine any fluctuation in the activation results and determine an acceptable window between blood collection and testing. Positive and negative controls must always be included in the BAT assay as high background activation could lead to false positives and unresponsiveness to anti-IgE stimulation indicating a non-responder in the assay could lead to false negatives. The MAT assay has an advantage in that cells are primed with plasma or serum and thus timing of the assay is not a major constraint as in the BAT. Ultimately, a coefficient of variation (CV) will need to be developed for replicates performed in BAT and MAT assays. These aspects of technical validation are being explored now^{13, 26} and are not seen as a major hurdle in implementing BAT and MAT for eventual clinical use. Similar aspects need to be covered for BBEA.

3. Clinical validation

Once a diagnostic test method has been optimized and standardized and has shown to be consistent and reliable from a technical point-of-view, it needs to be clinically validated, i.e. it needs to be compared with the gold-standard for diagnosis to determine how the results should be interpreted in relation to the clinical phenotype. In the case of food allergy, the gold-standard to be used for comparison should ideally be DBPCFC. Depending on the results obtained for a given test in individuals with and without allergy, cut-offs can be defined to determine when a test should be considered positive and a non-dichotomous approach can

also be adopted in which the results can be associated with a certain probability of clinical food allergy.

Recently, a BBEA for the diagnosis of peanut allergy was validated for use in the United States using two independent cohorts from the CoFAR2 and POISED clinical trials²⁷. The peanut BBEA was found to correctly diagnose 93% of subjects, with 92% sensitivity, 94% specificity, 91% positive predictive value (PPV) and 95% negative predictive value (NPV). In other studies awaiting validation, the epitope profiling has been shown to correlate with the severity of reactions to peanut^{28, 29} and to distinguish milk-allergic children who will react to unprocessed milk, baked-milk or fermented milk products³⁰.

For BAT to peanut, Santos et al¹⁵ have identified the optimal cut-off which showed 97% overall accuracy, 98% sensitivity, 96% specificity, 95 PPV and 98% NPV for peanut allergy. Similarly, for sesame and tree nuts, optimal cut-offs were identified and demonstrated to be able to reduce the number of OFC, especially the number of positive OFC in which patients would have experienced an allergic reaction of varying severity¹⁶. Other studies have looked at the utility of BAT to support the diagnosis of allergy to other foods³¹⁻⁴¹.

As different food allergies can be quite distinct in terms of the phenotype of patients (e.g. in milk and egg allergies, an intermediate phenotype of patients tolerating baked milk/egg can be identified) and in terms of the nature of the allergen that IgE recognises, diagnostic tests can be interpreted differently and need to be validated for different food allergies. For example, 95%PPV cut-offs for SPT and sIgE to extracts and components are different for cow's milk, egg and peanut allergies^{6, 42}. Other factors that can influence the cut-offs and the predictive value of a given test are: the patient population, the geographical location and the clinical setting^{19, 43}. This explains the identification of different cut-offs for a given test, such as sIgE determination whose method is standardized and widely available, in different patient populations⁴⁴. We anticipate that thresholds to confirm clinical reactivity using BBEA, BAT and MAT will be different for varying foods.

4. External independent validation

As outlined in a publication by the Board on Health Care Services of the National Academy of Sciences⁴⁵, diagnostic tests must be rigorously developed and documented in accordance with national guidelines. As depicted in **Figure 3**, the major steps in test development include a test discovery stage, a validation stage, and an evaluation of clinical utility stage. In the discovery

stage, a large, well-characterized cohort of specific food-allergic patients is divided into two sets: a training set and a confirmation set. The training set is used to establish the diagnostic standard or algorithm, which is then “locked-down” prior to testing samples in the confirmation set. Unless the test performance of the confirmation set is in the range of the training set, it is likely that the diagnostic standard or algorithm has been “overfit” and will not be useful with other patient cohorts. Ideally, a second sample set from an independent cohort should be used, which will give a more realistic picture of how the diagnostic standard or algorithm will perform. The test can be further validated based on results of the analytic validation, but then “locked-down” prior to final assessment of clinical utility. External independent validation has been completed for both the BBEA²⁷ and the BAT^{15, 17} to diagnose peanut allergy. For instance, the optimal cut-off defined for BAT in the primary peanut study¹⁵ was externally validated in an independent cohort with 100% specificity and 100% PPV¹⁵. The same method for BAT was used in the LEAP and associated studies¹⁷, confirming the diagnostic value with the previously defined cut-off showing 99% specificity and 95%PPV.

5. *Practical and logistical issues*

The BAT assay requires peripheral blood, which needs to be processed soon after collection and this can raise practical issues with implementation into clinical practice. Another important consideration is transportation to the lab. Shipping at ambient temperature may not be ideal since fluctuations in environmental temperatures vary dramatically between seasons and geographic locations and may alter basophil reactivity. A possible solution is shipping under controlled temperatures using insulated boxes with elements that can regulate the temperature of the sample within a defined range to avoid variation with temperature extremes during transportation. An additional practical issue to consider is the use of anticoagulants. Heparinised blood is used in research settings for BAT assays and other tube types are also acceptable²². EDTA, however, may inhibit basophil degranulation due to calcium chelation and therefore calcium needs to be added before stimulation.

MAT and BBEA use plasma or serum, which can be stored over long periods of time and tested when optimal conditions are achieved, and thus the logistical aspects to consider are, for instance, the storage space of samples for both MAT and BBEE, and the maintenance of the cell line in culture in appropriate conditions for MAT. Using primary cells for passive sensitization assays have additional practical challenges related to the standardization and

normalization of results given the variability of cellular reactivity between donors and over time.

6. Regulatory approval

In general, three pathways are available to obtain final FDA approval of a test, as depicted in **Figure 4**⁴⁵. Ideally, the clinical utility of a test should be derived from a prospective clinical trial. However, this is not always practical or feasible, and in such cases, archived samples from prospective or retrospective studies may be utilized. If samples are derived from a prospective study, FDA consultation is typically obtained to determine whether an investigational device exemption (IDE) is required, i.e. in cases where the outcome of the test will influence patient management. Depending on the study design and outcome, the FDA may grant approval of the *in vitro* test for general use or designate it as a Laboratory Developed Test (LDT), which means the test is only approved for use in the single developing lab, prior to final approval. It should be noted that the FDA performs an in-depth review of the analytical and clinical validity of a test whereas the Centers for Medicare and Medicaid Services (CMS) regulates all laboratory testing in humans in the US through its CLIA program. CMS must also review and approve the performance characteristics of a test, e.g. accuracy, reportable range, reference interval, etc., prior to its availability for general clinical utilization.

Recommended clinical approach

1. Reality Check:

Obtaining final FDA and CMS approval in the US for an early-stage diagnostic is not unlike obtaining FDA approval for a new drug. Several phases of validation are required and can easily take 5 – 10 years. At the present time, there is one allergenic epitope-based diagnostic assay for peanut, VeriMAP Peanut® (AllerGenis, Hatfield, PA), which has 93% concordance with DBPCFCs and has achieved LDT status in the US. Full FDA approval may occur within the next 1 – 2 years. Full FDA and CMS approval for similar epitope-based assays for milk and egg will likely take another 3 – 5 years, and full approval for other assays discussed in this review can potentially take longer.

2. Indications

Given the improved accuracy over currently available diagnostic tests, the next generation of food allergy diagnostic tests should eliminate the need for most OFCs to confirm the diagnosis of food allergy. The BBEA has been independently validated to provide greater than 90% accuracy in diagnosing clinical peanut allergy. BAT has demonstrated 97% diagnostic accuracy in a patient population recruited from a specialized Allergy center covering a range of ages. In a subgroup of patients with equivocal diagnosis following history, SPT and sIgE to peanut and to Ara h 2, BAT had 93% accuracy¹⁵. MAT had ca. 88% accuracy in the same patient population¹⁸.

3. Severity/Threshold

Two studies using earlier microarray technology have evaluated the correlation between allergenic-epitope diversity and the severity of clinical reaction to peanut^{28, 29}. IgE diversity to sequential epitopes of Ara h 1, 2 and 3 correlated with reaction severity, as well as *in vitro* basophil cell degranulation. Studies are currently underway utilizing the BBEA and machine learning to develop predictive algorithms that should more precisely predict clinical sensitivity, i.e. eliciting dose, and clinical severity.

Various studies have shown that the proportion of activated basophils and mast cells in response to allergen is associated with severity of allergic reactions and with relatively low threshold of reactivity^{17, 18, 46-48}. Specifically, for peanut allergy, basophil activation above 48% identified subjects with severe and life-threatening reactions during peanut challenges with 100% sensitivity, 97% specificity, 41%PPV and 100% NPV. BAT above 1.7% identified the patients who reacted to 0.1g of peanut protein or less during the OFC with 95% sensitivity, 91% specificity, 43% PPV and 100% NPV¹⁷. An association between BAT and severity and threshold had also been established in earlier studies^{35, 37}.

The risk assessment of individual patients requires, however, a holistic approach which takes into account the clinical (e.g. age, presence of asthma and other co-morbidities) and psychosocial context (e.g. anxiety, risk taking behaviour, access to emergency services) of the patient and their family needs^{49, 50}.

4. Prediction of response to treatment

Once a test has been validated for diagnosis, it can potentially be used to follow response to treatment. The concept of monitoring treatment responses has become increasingly

important as approval of the first food immunotherapy, Palforzia, occurred in 2020 and will likely give rise to more widespread use of oral immunotherapy, at least in the case of peanut allergy. Other biomarkers, such as sIgE, IgG4, and SPT used to monitor oral immunotherapy have not been especially promising. BBEA was shown to predict which milk-allergic children will achieve sustained-unresponsiveness following oral immunotherapy,⁵¹ to predict infants at high risk who will go on to develop peanut allergy,⁵² and to predict the eliciting dose threshold of peanut allergic children (unpublished).

Several examples of BAT assays following the course of peanut, egg, and milk food-specific immunotherapy have been published and are associated with desensitization⁵³⁻⁵⁷. Similar suppression of basophil activation has been demonstrated following peanut sublingual and epicutaneous immunotherapy^{58, 59}, indicating that measuring BAT during immunotherapy may indicate a desensitized state. Considerably less is known about how the BAT test can be used to determine sustained unresponsiveness, however, early reports are promising. For example, Patil et al⁶⁰ have recently described different trajectories of basophil response to Ara h 2 in patients treated with peanut oral immunotherapy over time depending on their clinical response, either transient desensitization or sustained unresponsiveness. In this study, changes in basophil response as early as 3 months into the treatment could already predict which group in terms of clinical response to treatment patients would fall into. Similar findings were reported by Chinthrajah et al⁶¹. Based on selected baseline peptide-sIgE and IgG4 levels, Suarez-Farinas et al⁵¹ built predictive models to predict the likelihood of sustained unresponsiveness following milk-oral immunotherapy, which was more predictive than models based on IgE to allergen components from cow's milk. Similar findings were reported for peanut OIT⁶².

Conclusion

BBEA, BAT and MAT are the next generation of food allergy diagnostics, making the transition to clinical practice. With the potential to enable a more precise diagnosis and to reduce the need for OFC, it is important to gather the evidence required to pursue the next steps towards regulatory approval of these new tests and their incorporation into clinical practice and guidelines, for the benefit of patients with suspected food allergy.

Tables

Table 1. Diagnostic performance of next generation food allergy diagnostic tests

Dignostic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Epitope testing				
BBEA Peanut ²⁷	92%	94%	91%	95%
Basophil activation test				
BAT to Peanut ^{15, 17}	83-98%	96-100%	95-100%	83-98%
BAT to Cow's milk ³¹	91%	90%	81%	96%
BAT to Egg ³²	90%	100%	100%	92%
Mast cell activation test				
MAT to Peanut ¹⁸	75%	99%	95%	92%

Figures

Figure 1. Bead-Based Epitope Assay (BBEA): measures epitope-specific IgE and IgG4

Figure 2. Overview of the basophil activation test (BAT) and the mast cell activation test (MAT)

Figure 3. Test Discovery and Validation Pathway⁴⁵. Abbreviations: FDA, the Food and Drug Administration; IRB, Institutional Review Board

Figure 4. Evaluation of Clinical Utility and Pathway to FDA Approval⁴⁵. Abbreviations: FDA, the Food and Drug Administration; IDE, investigational device exemption; IRB, Institutional Review Board; LDT, laboratory developed test.

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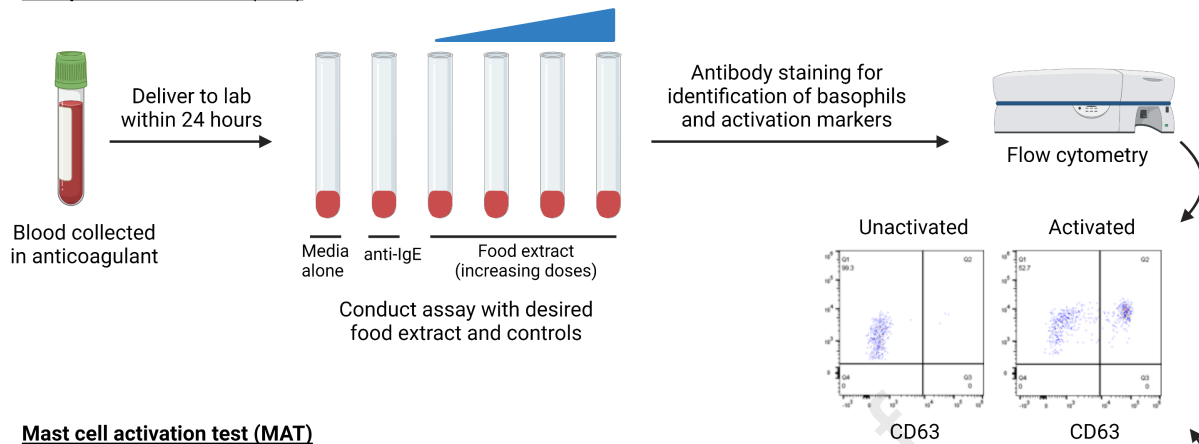
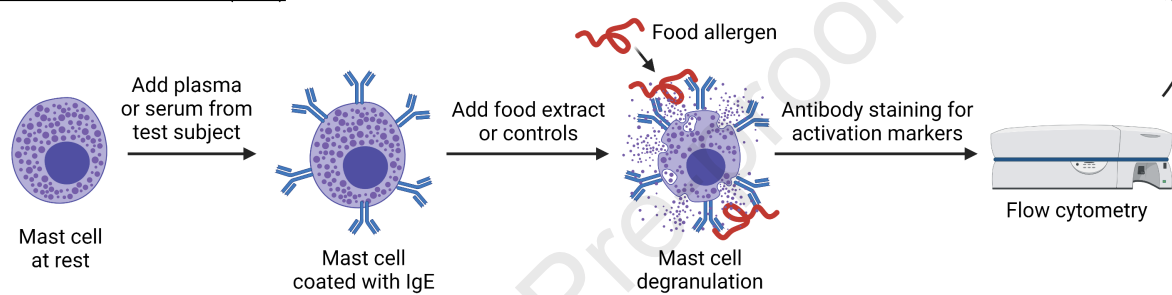
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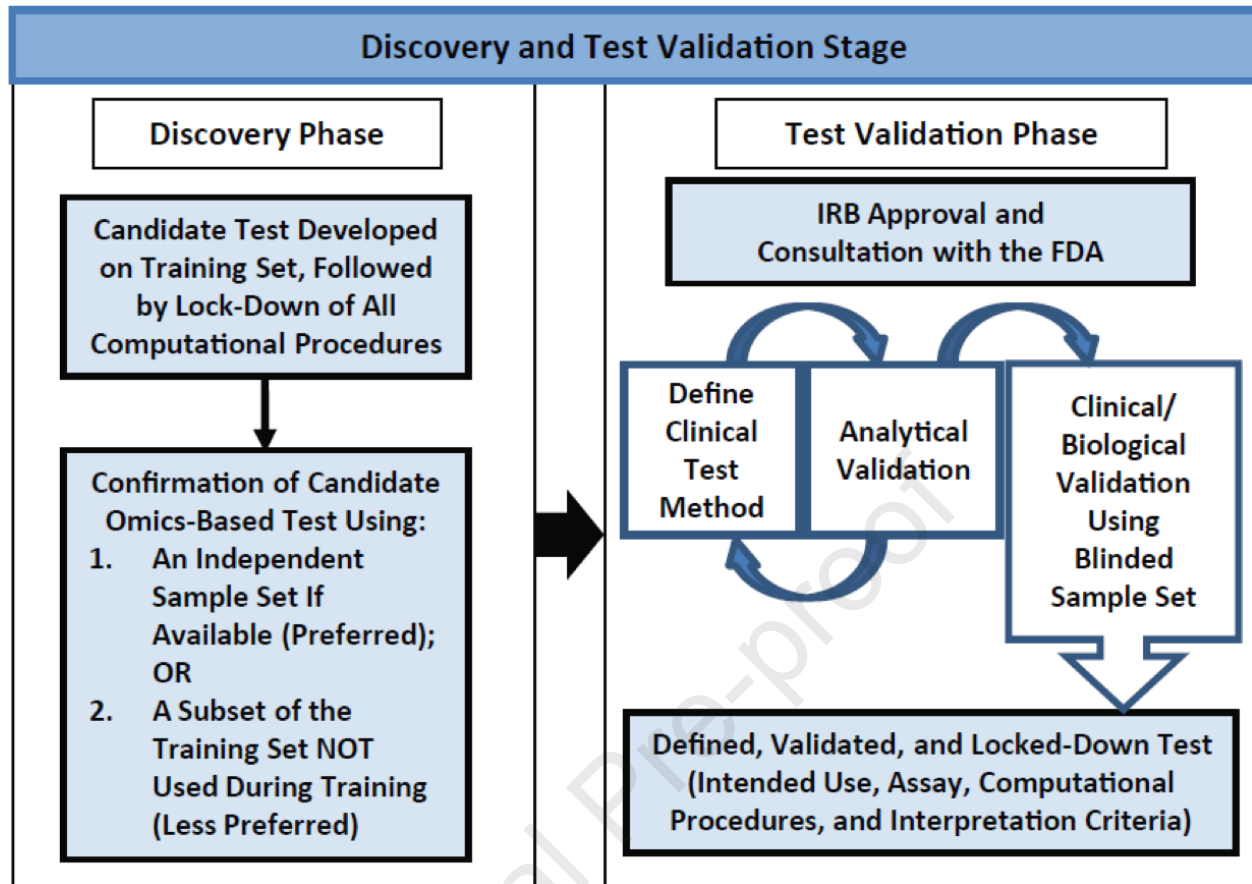
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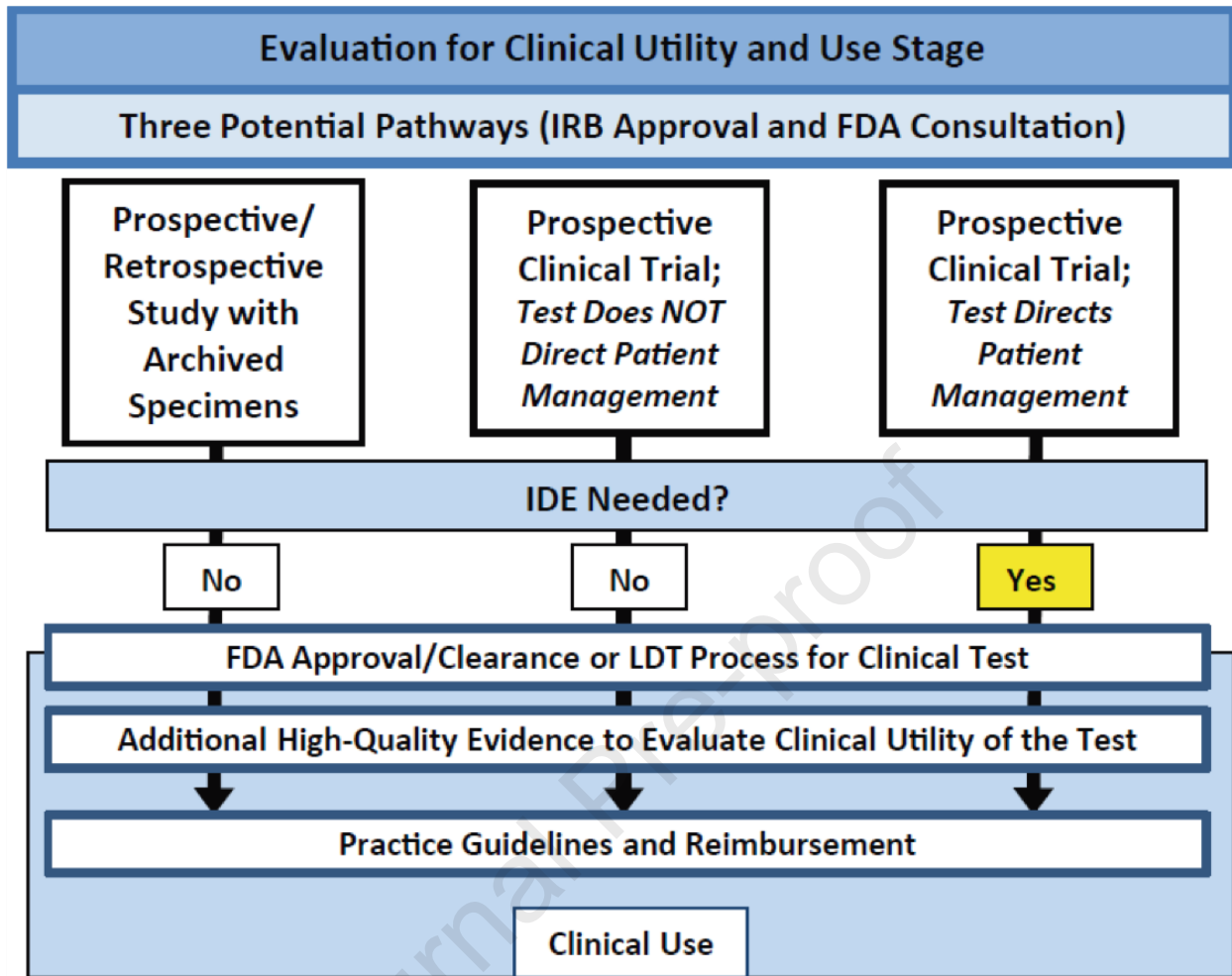
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Basophil activation test (BAT)**Mast cell activation test (MAT)**





CME EXAM

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Learning objectives:

1. To describe the principles of the basophil and mast cell activation tests and of determination of IgE to allergen epitopes.
2. To explain the diagnostic performance of the basophil and mast cell activation tests and IgE to allergen epitopes in food allergy.
3. To describe the added value of the basophil activation test and of the IgE to allergen epitopes to tests currently used in routine clinical practice.

Questions:

1. Which of the following is true regarding the bead-based epitope assay (BBEA)?
 - a. It requires collection of large volumes (milliliters) of blood.
 - b. It can use serum but not plasma.
 - c. It measures IgE to linear peptides.
 - d. It requires knowledge of the structure of the allergens.

Answer: C

Explanation: The BBFA is a multiplex assay that assesses IgE and IgG4 binding to allergen peptides based on their amino acid sequences representing various sequential epitopes.

2. Which of the following is true regarding the basophil activation test (BAT):
 - a. It assesses the function of IgE following stimulation with allergen.
 - b. It uses frozen blood samples.
 - c. It can use allergen extract but not single allergens.
 - d. It is less sensitive than the mast cell activation test using the LAD2 cell line.

Answer: A

Explanation: The BAT is a functional test that takes into account all characteristics of allergen-specific IgE (i.e. allergen-specific IgE levels, ratio allergen/total IgE, avidity and diversity) as well as cellular characteristics of basophils in their response to allergen stimulation.

3. Which of the following best describes the advantage of BBEA compared to standard determination of specific IgE to extracts or components?
- a. higher diagnostic accuracy
 - b. easier to perform
 - c. more accessible/available
 - d. fully quantitative method.

Answer: A

Explanation: Advantages of the BBEA include the microliter quantities of serum or plasma required to perform the assay, its technical ease of performance and reproducibility, its greater degree of accuracy, and its potential to predict clinical outcomes. SPT and specific IgE are easier to perform and are more readily available than the BBEA.

4. Which of the following is the greatest added value of the BAT compared to skin prick tests and allergen-specific IgE tests?
- a. higher sensitivity
 - b. higher specificity
 - c. more accessible/available
 - d. easier to perform

Answer: B

Explanation: SPT and specific IgE have high sensitivity but low specificity. BAT has higher specificity than SPT and specific IgE with retained high sensitivity. SPT and specific IgE are easier to perform and are more readily available than the BAT.