## Chapter 6

**Laboratory tests in the diagnosis of allergies**

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# Abstract

The term "allergy" refers to a hypersensitivity or disorder caused by an aberrant response to a particular immunological trigger, also known as an allergen. Despite rapid technological advancements, allergy tests aid in diagnosis only when taken into consideration along with clinical symptoms and history. The place where the complaints that are thought to be allergic are triggered (school, home, outdoor), time (seasonal, daily changes), climate changes (city, location change – seaside, inland or rural, urban area) or animal contact (cat, dog, bird, etc.) should be carefully questioned. Thus, the allergen to be investigated and the allergy tests to be used can be determined accurately. Allergy tests are used for the diagnosis and follow-up of allergic disease, determination of allergen protection methods, selection of specific allergen therapy (drug therapy or immunotherapy), and early identification of patients at high risk of developing allergic disease. There are numerous in vitro and in vivo diagnostic techniques available to identify allergies. In this section, under the title of in vitro tests which are tests performed on biological samples taken from the patient, IgE (total and allergen specific IgE), eosinophil count (in peripheral blood, nasal and induced sputum), and basophil activation tests …… will be mentioned.

200 words

# Keywords 3-6

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# Introduction

Allergic diseases are considered as the most prevalent chronic disease in Europe which affects about 150 million individuals. More than 50% of the population in Europe is predicted to experience an allergy by 2025 (Manifesto, 2015). In order to diagnose and treat patients with allergy, the clinical laboratory has a clear role to play. Increased blood levels of total IgE and/or allergen-specific IgE signify the occurrence of an IgE-mediated event. Similar information can be obtained using techniques like basophil degranulation and basophil or leukocyte histamine release. Histamine can be measured in whole blood or plasma using sensitive and exact procedures that can be automated. Urine tests can check for methyl histamine. In conditions like asthma and atopic dermatitis, eosinophil cationic protein levels in serum can be utilized as a marker of eosinophil activation. Increasing the level of eosinophil is a relatively non-specific finding. Serum mast cell tryptase levels, in a similar manner, can support or disprove an anaphylactic reaction both during life and as a cause of death (Salkie, 1994). Based on the patient's medical history, potential environmental triggers, and practical considerations (cost, anaphylaxis risk, necessary time), tests should be carefully chosen. Tests may be designed to measure functional and structural disabilities or to identify causes (Gupta et al., 2019).

Recently, in vitro testing has grown in significance in the process of diagnosing allergies. This diagnostic strategy lowers the risks associated with a potential cross-reactivity or clinical symptoms that do not match the sensitization data acquired in the laboratory while increasing the possibility of reaching a diagnosis with improved diagnostic specificity (Jakob et., 2015).

**Immunoglobulin E (IgE) determination**

In this test, the serum IgE level in a subject's blood sample is utilized either to determinetotal IgE (tIgE), regardless of specificity, or the allergen-specific IgE (As-IgE).

*Total IgE levels in serum*

Immunoglobulin E (IgE) was first described in 1967 as the fifth class of immunoglobulins that acts as a humoral indicator of the type 1 hypersensitivity response. It is found in the blood as a monomeric protein consisting of two heavy chains (ε chain) and two light chains, with a molecular weight of around 190 kilo Daltons (Johansson, 2014). A common way to describe the amount of the IgE molecules existing in serum is kilo units per liter (kU/L). According to Zetterstrom and Johansson, the typical reference value of total IgE levels in serum of non-atopic adults is1.5–114 kU/L (Zetterström & Johansson, 1981). Maternal IgE does not cross the placenta. Fetus can synthesize IgE from the 11th week of intrauterine life. Since there is no allergen warning, fetal IgE is expected to be low while in the uterus. IgE, which gradually rises in the first years of life from birth, peaks in adolescence. It then declines towards adulthood. In addition to age, gender, race, genetic characteristics, some non-allergic diseases, smoking, and allergen exposure may lead to an increase in IgE (Atkins & Leung, 2011).

In comparison to the general population, patients with allergic diseases such atopic dermatitis, asthma, and allergic rhinitis frequently have higher blood levels of immunoglobulin E (IgE). Although a patient's high total IgE may suggest that they have an atopic disorder, it is impossible to determine the sort of disorder or the type of allergens they are susceptible to. Furthermore, total IgE has limited usefulness in the diagnosis of these widespread illnesses due to the high degree of overlap in IgE levels in patients with and without allergic disease (Wittig et al., 1980).

Total IgE >417 kU/L is one of the diagnostic criteria. The specific disorders that are associated with very elevated levels of total IgE, are clinical situations in which the measurement of total IgE is useful are as follows (Kairemo et al., 1999; Bobinskas et al., 2015);

* Parasitic infections
* Rheumatoid arthritis
* Enfections such as allergic bronchopulmonary aspergillosis and Ebstein Barr virus infection
* Immunodeficiency disorders such as AIDS, hyperimmunoglobulin E syndrome, and Wiskott-Aldrich syndrome
* Malignancies and neoplastic disorders

It is also recommended to be used in the follow-up of the disease to evaluate the response to treatment and to detect exacerbations (Atkins & Leung, 2011).

The enzyme-linked immunosorbent test (ELISA) is often used to quantify total IgE by utilizing specific anti-IgE monoclonal antibodies.

## Allergen specific IgE

Specific IgE antibodies in a patient's serum are used in in vitro testing to bind to allergens that are linked to a solid phase. An enzyme-conjugated anti-IgE that causes a substrate to either change color or become fluorescent is then used to detect these IgE antibodies. The color change or fluorescence can then be measured and reported as units or nanograms per mL after being contrasted with the proper control responses. Although there are variations based on the specific technology employed, these enzyme immunoassays are sensitive and specific. Even though the original Radio-Immunosorbant Assay is no longer in use, it is still frequently used to refer to in vitro tests (.

**In vitro determination of IgE** (https://www.degruyter.com/document/doi/10.1515/almed-2020-0051/html)

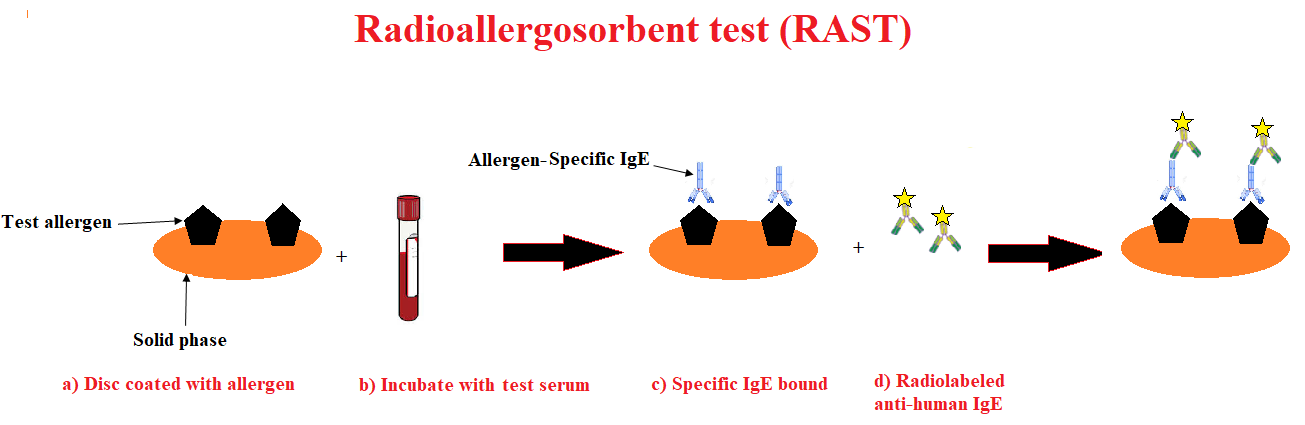
IgE molecules produced against specific individual antigens are labelled as serum specific IgE (sIgE). These were detected by Radio-Allergo-Sorbent Test (RAST) using radiolabeled (I125) antihuman IgE molecules. Radio-isotopes have now been replaced by enzyme conjugated antihuman IgE antibodies (Immunocap). The major pitfall of sIgE estimation is false positivity with high total IgE levels (>300 kIU/L) due to non-specific binding to test allergens [11]. (alergy testing--)

The most frequently requested in vitro allergy test among the numerous approaches to screen for allergies is detecting allergen-specific IgE (as-IgE) antibody, which is examined in serum by allergen-specific IgE (as-IgE) testing. The quality of the allergen component employed, the ability to assess the particular IgE specific to this component, and the level of confusion brought on by binding non-IgE isotypes all influence the characteristics of the tests to detect as-IgE (16). The tests allow for the measurement of qualitative variables (positive or negative results), semi-quantitative variables (class 0, I, II, III, IV, V, VI), and quantitative variables (kIU/L, kUA/L, or ng/ml). Quantitative tests have become more and more popular in recent years. The Food and Drug Administration (FDA) has approved three commercial test systems with the highest analytical sensitivity (0.1 kUA/L), but since each system measures different IgE populations or as it is impossible to compare the effectiveness of measuring IgE antibodies, the test must be conducted using various methods. Comparison of the outcomes is not feasible. Consequently, various as-IgE measurement findings for the same allergen from the same patient are produced using various methodologies. Therefore, each measurement should be performed using the same method if these tests are to be used in the follow-up of allergic patients, particularly for food allergies (17).

The first commercial method for detecting the as-IgE antibody was the Phadebas radioallergosorbent test (RAST), which was introduced into use in 1972. A RAST involves binding an allergen (antigen) to a solid phase, like a paper disk (Figure 1, a), then incubating it with human serum, so that as-IgE molecules in the serum are bound (Figure 1, b and c). Unbound serum proteins are removed by a buffer wash, and the quantity of as-IgE bound is then determined followed by the addition of radioisotope-labeled anti-IgE molecules (Figure 1, d). The signal that is emitted by the radioisotope-labeled antibody will enable the detection of the concentration of IgE (Casas et al., 2020).

IgE per milliliter of serum is an arbitrary unit used to report the results. RAST was once a brand name, but it is now frequently (and incorrectly) used in colloquial language to refer to any assay for as-IgE (Kim & O'Gorman 2004). These antibodies alone are adequate to foretell that an individual will experience an allergic reaction when exposed to the allergen. Although there may be a high level of antibodies in the blood, this does not always indicate how severe, if any, an allergic reaction will be.

This is used to interpolate results at kUA/L of sIgE, where one unit equals 2.4 ng of IgE. There is evidence that one unit (kUA/L) of sIgE is equivalent to one unit (kU/L) of tIgE [21]. (Jakob et., 2015).



Specific IgE (RAST) measures allergen-specific IgE to allergens in patient serum. In the case of inhalant allergens a level of>0.35 kU/l is considered positive (sensitivity 60- 80%, specificity 90%); for food allergy, the cut-off values for positive (indicating clinical reactivity) appear to be much higher (Fig. 1).10 The respective advantages of SPTs and specific IgE are shown in Table II. (Diagnostic testing in allergy--)

Over time, a more recent class of second-generation, as-IgE antibody clinical tests which are more sensitive and specific than earlier techniques have been developed. The ImmunoCap System™ (Therma Fisher, Upsala, Sweden) is a fully automated system for quantitative measurement of as-IgE in human serum or plasma. In this system, a cellulose sponge is utilized as a solid phaseis in order to increase the binding capacity to allergens and also fluorescent anti-IgE is applied in a quantitative fluorescence enzyme immunoassay.

ImmunoCAP™ and ImmunoCAP™ ISAC (Thermo Fisher Scientific); Immulite® (Siemens); Euroline® (Euroimmun); and ALEX®/ALEX2® (Macro Array Diagnostics) (Casas et al., 2020).

Several novel testing techniques based on the as-IgE antibody detection system have been developed recently as a result of technology advancements and a rising need for in vitro diagnostic tests. Compared to their predecessors, these tests are also more automated, quantitative, and reproducible (Makhija & O’Gorman 2012). The following tests are as (Casas et al., 2020; Sicherer & Wood 2012):

* The ImmunoCap System™ (Therma Fisher, Upsala, Sweden) is a fully automated system for quantitative measurement of as-IgE in human serum or plasma. In this system, a cellulose sponge is utilized as a solid phaseis in order to increase the binding capacity to allergens and also fluorescent anti-IgE is applied in a quantitative fluorescence enzyme immunoassay.
* Immulite® (Siemens Healthcare Diagnostics, Los Angeles, Canada)
* Euroline® (Euroimmun)
* ALEX®/ALEX2® (Macro Array Diagnostics)
* HYTEC-288 (Hycor Biomedical, Garden Grove, Canada)

Although all of these tests are based on the recognition of antigens by antibodies, they vary in terms of how allergens are bound, signal detection methods, quantification type, the necessary sample size, and automation degree [22], [23], [24], and [25]. The in vitro allergy test can be carried out using either a singleplex assay, which measures the presence of sIgE against a single allergen or allergenic source, or a multiplex assay, which evaluates the presence of sIgE against a battery of allergens concurrently (Lee et al., 2015).

Due to nonspecific binding of allergen to some immunosorbents, false positive results in individuals with high total IgE levels and falsely low results in patients with high levels of IgG antibody are drawbacks of in vitro allergen-specific IgE testing (Makhija & O’Gorman 2012).

Indeed, as-IgE and skin tests both detect the same thing. As-IgE tests cost more and produce long-lasting results. However, it is preferred to skin tests in the following unique circumstances:

- Previous history of a life-threatening reaction to an allergy

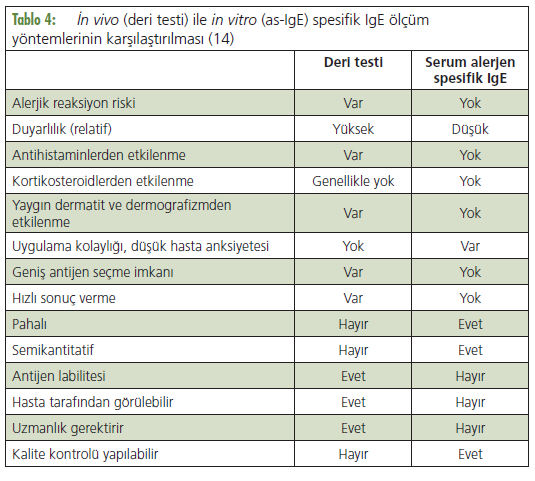
- Pathological skin disorders (active dermatitis, dermographism) in the area where the skin test will be applied.

- Within two weeks of experiencing a severe acute allergic reaction (at this time, all mast cell mediators are depleted, therefore skin testing may produce false negative results)

- In situations where antihistamines and tricyclic antidepressants, which will negatively affect skin test results, cannot be stopped.

- In situations where the patient refuses to cooperate (mental or physical problems).

The characteristics related to the skin test and serum as-IgE test are compared in Table 4.



(Eigenmann et al., 2013)

(Osguthorpe, 2014)

**Eosinophil Count**

Since eosinofils are considered as the main effector cells of allergic diseases which mediate cytotoxic and inflammatory processes in allergic disorders such as rhinitis, bronchial asthma, and urticaria, they are frequently found in increased levels called ‘eosinophilia’, in peripheral blood and related tissues (Gleich et al. 1993, Kroegel et al. 1994).

*Eosinophil levels in peripheral blood*

Following allergen exposure, eosinophilia (an eosinophil count exceeding 500 per microliter of peripheral blood or 4% of total leukocytes in blood) might ocur (Celakovska et al., 2019). Its diagnostic value is not high, as it is not necessarily observed in the context of allergic disorders. Likewise, the presence of eosinophilia does not always indicate allergic disease. In addition, in cases such as drug allergies and eosinophilic pneumonia, increased eosinophil density can be detected in the relevant tissues, although there is no eosinophilia in the peripheral blood (Gupta et al., 2019).

*Nasal eosinophilia*

Eosinophil counts in nasal smear are an insensitive but moderately specific test for the diagnosis of allergic rhinitis compared to the presence of eosinophilia in the peripheral blood and allows to distinguish it from other causes of rhinitis. It is also a good indicator of response to topical nasal steroids. However, its use has been abandoned today due to the difficulty of the technique and lack of sufficient specificity and sensitivity (Rudrappa et al., 2019; Sonawane et al., 2016).

*Eosinophilia in sputum*

As an effective indicator of airway inflammation, sputum eosinophil count can be used to assess disease severity, treatment outcome, and prognosis in asthma patients (Bandyopadhyay et al., 2013). Patients with allergic asthma have been shown to have elevated eosinophil levels in sputum (Gupta et al., 2019).

## Basophil Activation Test

The basophil activation test (BAT) is a functional in vitro diagnostic assay assessing the level of degranulation of basophils using histamine and other mediator release. In this test, flow cytometry is utilized for measuring the expression of activation markers (such as CD63, CD203c), which are upregulated on the surface of basophils as a result of the cross-linking of IgE antibodies bound to the high-affinity IgE receptor (FcRI) as a result of allergen or anti-IgE stimulation (Dona et al., 2021; Hemmings et al., 2018; Santos et al., 2021). Despite numerous benefits of the BAT test, it still has significant drawbacks that prevent it from being used more widely. The BAT analysis is preferable to be performed within 4 hours after collecting the sample and the patients with non-responsive cells are not diagnosed any further using the procedure.

Mast Cell Activation Test

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