

Review



China Consensus Document on Allergy Diagnostics

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The consensus represents a joint initiative by the following associations: Allergy Prevention and Control Committee, Chinese Preventive Medicine Association; Allergy Medicine Committee, Chinese Research Hospital Association; Chinese Society of Allergy; Allergy diagnostics Subgroup, Allergy Prevention and Control Committee, Chinese Preventive Medicine Association; Allergy Prevention and Control Committee, Guangdong Preventive Medicine Association.























ABSTRACT

The prevalence of allergic diseases has increased dramatically in recent years in China, affecting the quality of life in 40% of the population. The identification of allergens is the key to the diagnosis of allergic diseases. Presently, several methods of allergy diagnostics are available in China, but they have not been standardized. Additionally, cross-sensitization and co-sensitization make allergy diagnostics even more complicated. Based on 4 aspects of allergic disease (mechanism, diagnosis procedures, allergen detection *in vivo* and *in vitro* as well as the distribution map of the most important airborne allergens in China) and by referring to the consensus of the European Society of Allergy and Clinical Immunology, the World Allergy Organization, and the important literature on allergy diagnostics in China in recent years, we drafted this consensus of allergy diagnostics with Chinese characteristics. It

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aims to standardize the diagnostic methods of allergens and provides a reference for health care givers. The current document was prepared by a panel of experts from the main stream of professional allergy associations in China.

Keywords: Allergen; diagnosis; consensus; China

INTRODUCTION

Allergic diseases are common, and there is a need to prevent and cure these diseases worldwide in the 21st century according to the World Health Organization (WHO). Allergic rhinitis (AR), a key subgroup of allergic diseases, affects nearly 40% of the world's population.¹ Data from epidemiological surveys in China have shown that the prevalence of AR increased by 6.5% from 2005 to 2011 and that the number of asthmatic patients also increased yearly.² Recent survey data from China have indicated that the prevalence of asthma in individuals aged 20 years or older is 4.2%.³ Over the past 30 years, the asthma prevalence in children in China has grown by nearly 150%, from approximately 2% in 1990 to more than 3% in 2020.⁴

AR is closely related to asthma. Almost 40% of patients with AR have asthma.^{5,6} In China, almost half of patients with pollen-induced AR in the summer and autumn can develop seasonal allergic asthma within 9 years; additionally, 90% of AR patients can develop asthma within 11 years.⁷ A 2009 national multicenter epidemiological survey in China showed that the main allergens leading to asthma were dust mites, cockroaches, pollen, and mold.⁸ Among these allergens, mold induces the most severe asthmatic reactions, which commonly progress to allergic bronchopulmonary aspergillosis (ABPA), a latent and easily misdiagnosed disorder that can cause irreversible damage to lung tissue and function in patients.⁹

Food allergy (FA) has also increased with the increasingly rich and diverse food structure and environmental factors. Studies by Chinese researchers have shown that FA accounts for 77% of patients with anaphylactic shock¹⁰; wheat-dependent exercise appears to be a primary cause of the life-threatening allergic reaction. The diagnosis of food allergens is further complicated by cross-sensitization with airborne allergens. For example, fruits and vegetables have cross-sensitization with various forms of pollen. Similarly, shrimp and crab show cross-sensitization with dust mites and cockroach allergens. This high level of cross-sensitization makes the diagnosis of allergic sensitivity more complex.¹¹

Allergy diagnostics is the core of the prevention and treatment of allergic diseases. Early diagnosis and treatment can effectively control disease progression, thereby reducing the pain and economic burden on patients. Therefore, improving the diagnostic testing of allergens can clarify the risk factors leading to allergic disease and provide a level of environmental control to improve the treatment of affected patients.

The number of patients with allergic diseases is increasing in China. Additionally, various diagnostic methods are creating confusion with no available standard. Thus, the Allergy Prevention and Control Committee, Chinese Preventive Medicine Association and the Allergy Medicine Committee, Chinese Research Hospital Association convened experts to discuss the issue. The associations reached a consensus on various aspects, including the general principles of allergy diagnostics and use of *in vivo* and *in vitro* allergen tests; the consensus was based on relevant information available from the European Academy of Allergy & Clinical

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Immunology (EAACI), World Allergy Organization and other recent evidence on allergy diagnostics available from recent domestic and international literature.

PATHOGENESIS OF ALLERGY

Allergy diagnostics strictly follows the pathogenesis of allergic diseases. Presently, 3 types of immune mechanisms are involved in allergic diseases: immunoglobulin E (IgE)-mediated, mixed (IgE/non-IgE), and non-IgE-mediated allergy.¹²

Mechanism of IgE-mediated allergy

IgE-mediated allergy primarily occurs through sequential phases involving sensitization to an allergen and subsequent challenge that stimulates a latent immune response. During the sensitization phase, allergens are processed and presented in the form of the major histocompatibility complex II-antigen-peptide complex by antigen presenting cells, most importantly dendritic cells (DCs). The antigen-peptide is then recognized by specific T-cell receptors on naïve CD4⁺ T cells, which proliferate and differentiate into various subsets of T helper (Th) cells, including Th2 cells. Th2 cells produce interleukin (IL)-4, IL-5, and IL-13. At the same time, cytokines and co-stimulating factors are released from stimulated DCs, and the expression of the latter is crucial for activating naïve T cells.¹³ With the help of Th2 cells, antigen-specific B lymphocytes differentiate into plasma cells capable of producing IgE. Secreted IgE binds to high-affinity IgE receptors (also known as FcεRI) on the surface of mast cells and basophils, causing these cells to be sensitized to specific allergens.

During the challenge phase, the primed immune cells are exposed to the same allergen which cross-links IgE–FcεRI complexes on the surface of sensitized cells. These activated cells degranulate and release vasoactive substances (histamine and other inflammatory chemical mediators) that increase smooth muscle contraction, stimulate mucus secretion, lower blood pressure, and result in tissue damage. This is the early phase response (EPR) that occurs within minutes after exposure to the allergen and lasts for 30–60 minutes. EPR is followed by the late phase response, which occurs 2–4 hours after stimulation and can last for 1–2 days or longer. In addition to mast cells and basophils, various other inflammatory cells are also involved in this response. For example, neutrophils, eosinophils, and macrophages migrate to the allergen-exposed site,¹⁴ secreting biologically active substances and enzymes that generate many of the symptoms involved in allergic disease.

Mechanism of non-IgE-mediated allergy

The mechanism of non-IgE-mediated allergy is less clearly defined. It has been proposed that the activation of complements leads to the production of anaphylatoxins such as complement 3a and complement 5a. These molecules can bind to corresponding receptors to facilitate smooth muscle contraction and increase vascular permeability. Neuropeptides, including substance P, vasoactive intestinal peptide, and somatostatin, strongly induce the release of mediators, especially histamine. Similarly, the mechanism of allergic reactions caused by non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, is not completely understood. Agents like opioids that prompt mast cells to secrete mediators are thought to cause the rapid release of histamine. Immunoglobulin G (IgG) and Fc-γ receptors may also play a role in the pathogenesis of this allergic reaction.¹⁵

STANDARDIZATION OF ALLERGY DIAGNOSTICS PROCESS

Currently, allergen testing mainly focuses on IgE-mediated type I hypersensitivity and T cell-mediated type IV hypersensitivity. Allergen tests are divided into 2 categories: (1) *in vivo* allergen tests including skin prick test (SPT), intradermal test (IDT), patch test, and provocation test; and (2) *in vitro* allergen tests including the serum allergen-specific IgE (sIgE) test, total IgE (tIgE) test, and basophil activation test (BAT). In the clinical diagnosis and treatment process, it is often necessary to combine both *in vivo* and *in vitro* tests, along with clinical history, to determine the type of allergen and the degree of sensitization as well as their relationship with disease symptoms, reducing the economic burden on patients caused by unneeded tests.

Allergic diseases are mainly diagnosed on the basis of clinical symptoms, medical history, physical examination, and allergen tests. **Fig. 1** illustrates the diagnostic flowchart of allergic diseases.

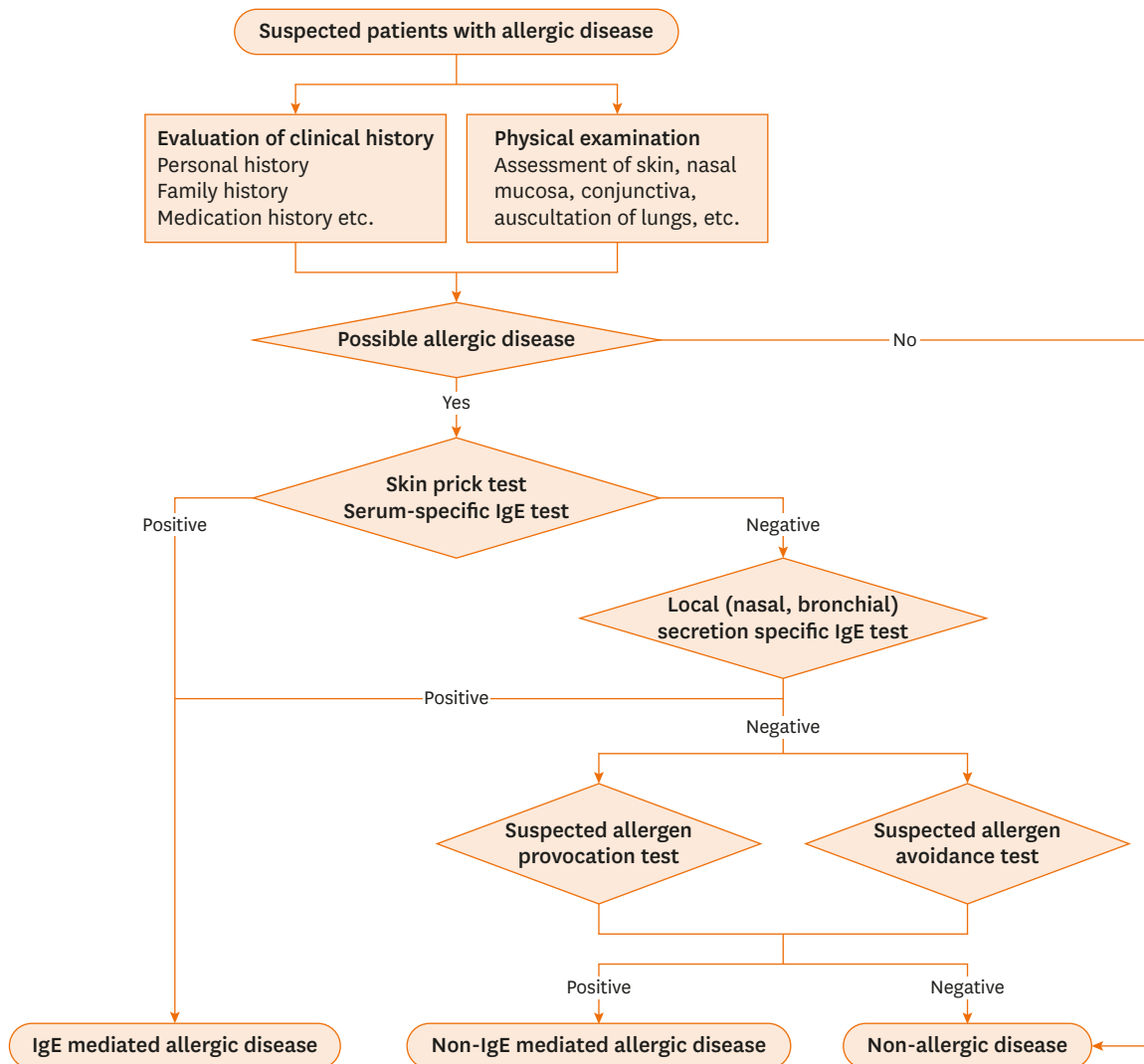


Fig. 1. Diagnostic flowchart of allergic diseases.
IgE, immunoglobulin E.

Table 1. Key points about symptoms and medical history

Key points
1. Analyzing the frequency and/or severity based on symptoms described by the patients
2. Personal or family history of asthma, rhinitis and eczema
3. Seasonal or perennial symptoms
4. Possible allergens in the home (e.g., dust mites, cockroaches, pets, and mould)
5. Occupation and hobbies of patients, particularly those related to allergens
6. History of food allergies and adverse drug reactions
7. Possible triggers
8. Impact of symptoms on lifestyle (i.e., work or study, sleep, social and physical activities)

Two key aspects are important when verifying the medical history of the patient: (1) whether an allergic disease is present and (2) which allergens may be related to the clinical symptoms of the disease (**Table 1**).

Physical examination of patients with suspected allergic diseases should concentrate on skin and mucous membrane such as the nasal mucosa, conjunctiva, and respiratory tract. Some special auxiliary tests, such as fractional exhaled nitric oxide, have good diagnostic value for allergic inflammation in the bronchi and nasal mucosa,¹⁶ which should be considered in allergic respiratory diseases (e.g., asthma and AR).

Allergy diagnosis can be achieved by *in vivo* and *in vitro* testing. *In vivo* approaches include skin test, allergen provocation test (APT), and patch test. *In vitro* tests primarily comprise allergen sIgE and specific IG4 (sIgG4) tests, but can also include other analyses such as BAT. Clinical history inquiry and physical examination are prerequisites for *in vivo* diagnosis, while standardized reagents and instruments are the cornerstones of *in vivo* tests.

In vivo allergen tests

Skin test

1. SPT

SPT is an important method to determine the cause of allergic diseases. It has high sensitivity, but the specificity is not so high as that of sIgE *in vitro* test.¹ The reliability of SPT depends on whether the allergen skin test solution has an appropriate allergen concentration and on whether it contains all the major sensitized components of the allergen. Manufacturers should clearly indicate the concentration, expiration date, and storage conditions (e.g., 2°C–8°C) on the label.

Tests should be applied to the volar aspect of the forearm, at least 2–3 cm away from the antecubital fossa. The back can also be used for SPT, particularly in infants and children. Small drops each of the allergen extract (prepared by manufacturers and the concentration labelled), histamine (positive control), and physiological saline (negative control) are placed successively on the surface of skin pre-sterilized with appropriate alcohol solution at intervals of more than 2 cm (**Fig. 2**). A special lancet is utilized to pierce the surface of the skin at the center of the allergen droplets, thereby penetrating the epithelial layer without inducing bleeding. To avoid cross-contamination and misjudgment, the reuse of lancets is not recommended. After 1 minute, excess droplets should be wiped away with filter paper. Practitioners should then measure the size of the wheal at the skin test site after 15 to 20 minutes. Traditionally, the longest diameter and its longest vertical diameter are recorded, excluding measurement of any pseudopod formations.

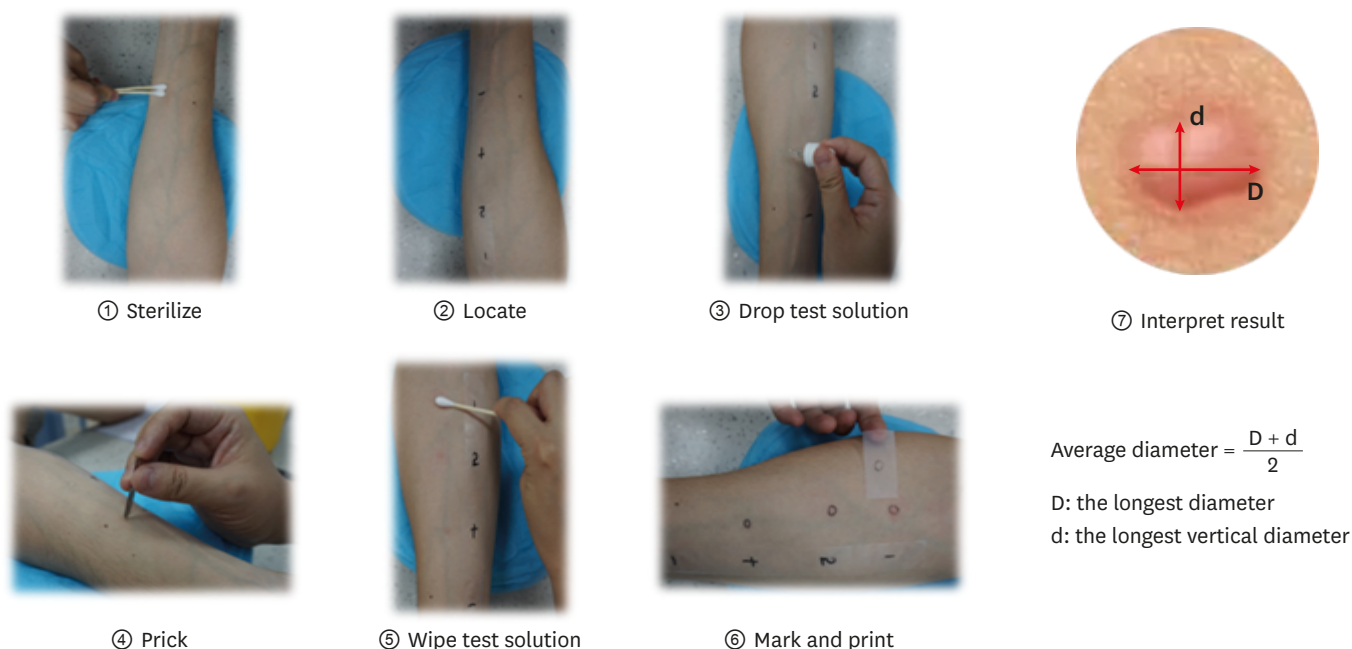


Fig. 2. Procedures of skin prick test.

In the absence of commercially available food allergen extracts, a prick to prick test (PPT) of fresh food can be used to diagnose FA. Although the PPT cannot be strictly standardized because it is performed with fresh food after simple processing, the procedure and interpretation of the results are similar to that of SPT. Severe allergic reactions created by PPT can occur¹⁷; the utilization of PPT must be closely observed in clinical application to minimize adverse reactions.

The indications,¹⁸ contraindications,¹⁹ precautions, and result judgment of SPT are shown in **Table 2**.

1) Results Interpretation

A positive result of a SPT only demonstrates sensitization to a specific allergen, but does not necessarily imply that the individual will have symptoms when exposed to that allergen. The

Table 2. Indications, contraindications, precautions and result judgment of SPT

Indications	Type I hypersensitivity, such as allergic rhinitis, allergic asthma, allergic conjunctivitis, food allergy, and insect venom allergy.
Contraindications	<ol style="list-style-type: none"> 1. Patients at high risk of severe allergic reactions: for example, patients with uncontrolled asthma, the emergence of severe allergic reactions after exposure to very small amounts of allergens, or a history of allergic shock within 30 days. 2. Patients with poor skin conditions, such as acute attack of urticaria, diffuse skin diseases including generalised eczema and mastocytosis, and severe dermatographism. 3. Other factors: patients are using medications that may affect the results or influence the use of epinephrine, such as antihistamines, beta blocker or angiotensin converting enzyme-inhibitor. 4. Relative contraindications: history of cardiovascular diseases, pregnancy, etc.
Precautions	<ol style="list-style-type: none"> 1. Standardized allergen extracts should be used to perform SPT, and all extracts must be stored at 2°C–8°C. 2. The test should include both a positive and a negative control. Histamine dihydrochloride (10 mg/mL) can be used as a positive control. Normal saline (0.9%) or allergen solvent can be used as a negative control. 3. When performing the test, the drops of 2 allergens should be placed at least 2 cm apart to prevent cross-contamination. After skin puncture, the location of each allergen should be marked. Excessive extract should be wiped away carefully, so that other test sites will not be affected. 4. The skin should not be pricked too hard (resulting in bleeding) or too lightly (without penetrating the epidermis).
Result judgment	<ol style="list-style-type: none"> 1. It is regarded as positive when the average diameter of wheals is greater than 3 mm, compared with that of the negative control. 2. The SI can be used to help determine the rank of the positive reaction. It is calculated as the ratio of the average of allergen wheal size to that of positive control wheal size. SI is expressed as: +, $0.3 \leq SI < 0.5$; ++, $0.5 \leq SI < 1.0$; +++, $1.0 \leq SI < 2.0$; +++, $SI \geq 2.0$.

SPT, skin prick test; SI, skin index.

wheal size reflects the level of sensitivity of the patient to the allergen, but the results are not completely correlated with clinical allergy symptoms. The accuracy of SPT results is affected by many factors, for example, the use of antihistamines, low potency of allergen extracts, and insufficient penetration of the skin by the lancet, which can lead to false-negative results and dermographism which causes false-positive results.

2. IDT

If SPT is negative and suspected allergens cannot be ruled out, the allergen IDT may be performed. IDT can better evaluate skin sensitivity to the low potency of allergen extracts and is more sensitive to diagnostic drugs and insect venom allergy than SPT (the comparison of SPT and IDT is shown in **Table 3**).

IDT is an intradermal skin test that uses a disposable 1-mL syringe with a 26 to 30 gauge needle to inject allergen extract solution (usually 0.02–0.05 mL) to produce a bulge of 2–3 mm in the dermis. Before injection, all the syringes should be carefully vented of air bubbles. The angle between the syringe and the skin is 45 degrees, and the bevel of the syringe needle should face the skin. Allergen extract concentrations used in IDT is usually 1/100–1/1,000 of SPT concentrations.²⁰ IDT is often performed on the volar side of the forearm, but not on the patient's back, and requires positive and negative controls. The indications for and contraindications into IDT are similar to those of SPT.

1) Results interpretation

The IDT results can be interpreted within 15–20 minutes after injection. Similar to SPT, the average value of the longest diameter and its longest vertical diameter of the wheal are used for interpretation. A wheal with a diameter greater than the negative control wheal indicates the presence of allergic sensitization. However, given the high sensitivity of IDT, positive reactions with small diameters may not have clinical significance. A positive response is defined as either a wheal that is at least 5 mm in diameter or 3 mm greater than the negative control wheal. The diameter of erythema should also be recorded. For example, in the US, the allergen biological activity index is determined by the size of erythema.²¹

2) Precautions

Although IDT can be performed by nurses or technicians, we recommend that a professional allergist be present. The factors affecting IDT are similar to those affecting SPT, but severe systemic reactions occur more commonly than SPT. When performing IDT, an adequate

Table 3. Comparison of SPT and IDT

Advantages	SPT	IDT
Ease of use	+++	++
Quickness	++++	++
Extent of reaction	++++	++
Adverse reaction	+	+++
False positive	Few	Possible
False negative	Possible	Few
Reproducibility	+++	++++
Sensitivity	+++	++++
Specificity	++++	+++
sigE antibody detection	Yes	Yes
Safety	++++	++
Use in infants	Yes	Unlikely

+, low; ++, moderate; +++, high; +++++, very high, SPT, skin prick test; IDT, intradermal test; sigE, specific immunoglobulin E.

emergency plan must be prepared. If severe local or systemic adverse reactions occur, ligation should be conducted using a tourniquet at the proximal end of the injection site, and 0.2–0.5 mL of 1:1,000 adrenaline should be injected intramuscularly into the contralateral arm. For patients with suspected drug allergy (DA), it is recommended to perform SPT before IDT.

3) Limitations to skin tests

There are some limitations to allergen skin tests. First, allergen extracts are complex mixtures of many proteins that are derived from diverse natural raw materials. These extracts are prone to change in chemical composition based on the growth conditions (*e.g.*, nutrients, growth temperature, and climate), genetic diversity of the species, and impact of other external environmental factors (*e.g.*, material age and extraction method) which can influence the source material and lead to differences between various lots of material and between various manufacturers.²²⁻²⁴

Secondly, the potency and concentration of allergen also affect the SPT result. Currently, the internationally recognized potency of allergen extract is mainly expressed in micrograms (μg) of allergens, because this is closely related to the overall biopotency of the extract. However, procedures for generating a given extract differ among manufacturers, making it impossible to compare between different products.^{25,26}

Thirdly, the potency of allergen extracts decreases with time and under high temperature. To ensure stability, allergen extracts are usually prepared with 50% glycerol as an adjuvant with suggested storage at 4°C.²⁶ Recombinant allergens are more stable than natural allergens, show better specificity, safety, and biological activity, and have a promising future in *in vivo* diagnosis. Nevertheless, the sensitivity of skin tests appears to be lower using recombinant allergens than using natural allergen extracts.^{23,27}

Finally, other external factors can also affect the results of allergen skin tests. For example, SPT is affected by patient age. Children, particularly those under 2 years of age, are less reactive than adults²⁸; the wheal size of positive SPT increases in young people aged around 20 years of age, but decreases in people aged older than 60 years.²⁹ The test results are also related to the anatomical position of skin puncture, and the degree of reactions in different positions is as follows: middle and upper back > lower back > upper arm > elbow > forearm (ulnar side > radial side) > wrist.²⁹ Other than age and anatomical location, additional biological and physiological factors may also affect skin test results, such as skin histological properties (vascular and quantity), histamine receptors, mast cells, and skin thickness.³⁰

It must be emphasized that the outcomes of both SPT and IDT are affected by some medications (**Table 4**), particularly oral antihistamines, antidepressants, and topical corticosteroids; thus, medication history should be inquired in detail before skin tests.³¹

APT

APT originated from studies on AR. In the test, small amounts of allergens are applied to the mucous membrane of the body to simulate the natural onset of disease and trigger symptoms. The type of allergy and suspected allergens are then determined. Depending on the affected site of patients, APT for different organs can be performed. Commonly used tests include the allergen bronchial provocation test (ABPT), allergen nasal mucosal provocation test (ANPT), allergen conjunctival provocation test (ACPT), food provocation test (FPT), and drug provocation test (DPT). APT can be further performed in the following

Table 4. Recommended medication withdrawal time before SPT

Medications	Degree	Withdrawal time	Clinical significance
Oral H1-antihistamine	++++	2–7 days	Yes
Intranasal H1-antihistamine/H2-antihistamine	0 to +		None
Imipramine	++++	Up to 21 days	Yes
Phenothiazines	+ to ++	Up to 10 days	Yes
Corticosteroids			
Systemic, short term	0		None
Systemic, long term	Possible		None
Inhaled	0		None
Topical skin	+ to ++	Up to 7 days	Yes
Dopamine	+		None
Clonidine	++		None
Montelukast	0		None
Specific immunotherapy	0 to ++		None
Treatment of UV or PUVA	+++	Up to 4 weeks	Yes

SPT, skin prick test; UV, ultraviolet; PUVA, treatment of psoralen combined with ultraviolet A.

cases: evaluation of highly suspected allergic disease (such as local AR) in patients without positive results by the skin test and *in vitro* sIgE test; determination of the disease-causing allergens in patients with several known allergens; evaluation of the tolerance of allergic asthmatic patients before immunotherapy; and non-IgE-mediated FA.

1. ABPT

ABPT is a specific challenge test, during which mild symptoms of bronchial asthma are induced in patients by inhaling small amounts of allergens.^{32,33} Depending on the site of ABPT, it can be classified as indoor ABPT (performed in a laboratory) or occupational (on-site) ABPT (performed at the workplace).

ABPT can be used to evaluate the efficacy of treatment in asthmatic patients and to screen for suspected occupational or environmental exposure allergens. Subjects undergoing ABPT must meet the following criteria³⁴: (1) those who were diagnosed with asthma should be mild and stable; (2) they show positive results by SPT or sIgE, or are suspected of exposure to occupational/environmental allergens; (3) they are stable in airway non-specific reactivity (cumulative provocation concentration [PC] or provocation dose [PD] of methacholine [Mch] or histamine [His] when forced expiratory volume in 1 second [FEV1] decreases to 20% of the baseline, represented by PC/PD₂₀-Mch/His); (4) they understand SPT and are cooperative; and (5) they are aged between 18 and 55 years, because the response to allergen challenge will decrease or influencing factors will increase with age.³⁵

Airborne allergens are often associated with asthma. Common airborne allergens include pollen (*e.g.*, tree and grass), animal dander (*e.g.*, cat and dog), insects/parasites and their secretions (*e.g.*, mite and cockroach), and fungi (*e.g.*, *Alternaria* and *Aspergillus fumigatus*). Regardless of the type of allergen, a standardized allergen solution should be used in ABPT. If the subjects are positive to multiple allergens, they should be provoked by the allergen with the most frequent exposure in the environment or with the strongest skin test response. ABPT should be ideally performed outside the relevant allergen exposure or season.

Presently, ABPT is primarily administered by compressed air-/oxygen-driven aerosol inhalation. Common inhalation methods include 2-minute tidal breathing and multiple deep breathing.³⁶ The aerosol is administered by dose-escalation (2-fold, 5-fold, and even 10-fold increments), or a single dose/concentration, or continuous low dose/concentration.

ABPT should start with a low concentration for patients at the first time to take ABPT or occupational allergen screen. The initial allergen concentration should be as low as possible, or one causing a wheal greater than 3 mm in skin test, to ensure the safety of subjects.³⁶ The initial concentration for ABPT can also be calculated by the estimated formula, combined with the subject's non-specific airway reactivity and sensitivity to allergen (2-mm wheal caused by SPT).³⁷ If the same patient will undergo ABPT again, a concentration 2–3 increments lower than the previous PC₂₀ or the previous PC₂₀ should be used as the initial concentration. However, the state of the subject needs to be assessed and closely monitored before and during ABPT.

When performing ABPT, the dose of inhaled allergens is increased at 10-minute intervals until a significant decrease in FEV1 (usually a >20% reduction from baseline) occurs. The decrease in FEV1 at this time is called an immediate response. FEV1 reaches the maximum fall within 30 minutes after allergen inhalation, and returns to baseline within 2 hours. About 50% of people with an early response will experience a second fall in FEV1, which starts to decline within 3 hours after allergen exposure and reaches the maximum fall at 6–7 hours. This is called a late response. FEV1 returns to baseline within 24 hours.³⁸

The safety of subjects is most important in ABPT.³³ First, all the necessary medical equipment should be available, and the operator must be on site throughout the testing process and supervised by corresponding medically responsible staff. Secondly, standard operating procedures must be strictly followed during ABPT, required not only for the safety of subjects, but also for scientific and rigorous interpretation of test results. Thirdly, if subjects are allowed to leave the hospital about 8 hours after receiving ABPT, they must be informed of the treatment measures when obvious respiratory symptoms appear and leave with contact information on the medical staff to obtain emergency services at any time.

2. ANPT

ANPT reproduces an allergic reaction of the nose by applying allergens directly to the nasal mucosa. Positive symptoms include nasal pruritus, sneezing, runny nose, and turbinate edema as well as increased nasal resistance and extra nasal symptoms such as itchy eyes, lacrimation, and itching of the upper palate. ANPT is the gold standard for the diagnosis of AR. For local AR patients with negative results from SPT and serum sIgE, ANPT is an optimal diagnostic method. Patients undergoing ANPT need to be asymptomatic or have only mild symptoms without interfering with the test results. ANPT is not recommended during the acute episode of AR and should be performed 2–4 weeks after the episode.

Some medications that may affect the results should be discontinued before ANPT. For example, oral antihistamines should be discontinued for 48 hours to 1–2 weeks, topical antihistamines for 4–5 days, intranasal corticosteroids for 48–72 hours, oral corticosteroids for 2–3 weeks, cromolyn sodium for 1–3 weeks, nasal decongestants for 2 days, tricyclic antidepressants for 2–3 weeks, NSAIDs for 1 week, and antihypertensive medication such as reserpine or clonidine for 3 weeks. Additionally, patients must not smoke and drink within 24–48 hours before ANPT. The test should be postponed for 4 weeks if a viral or bacterial respiratory infection occurs or for 6–8 weeks after nasal surgery, and should be avoided during pregnancy.³³

Although ANPT is reported to be less risky, it is not recommended for patients with uncontrolled asthma or severe chronic obstructive pulmonary disease, those with

cardiopulmonary disease who cannot receive adrenaline, or those with a perforated nasal septum, severely deviated septum, or complete nasal obstruction.

The dose of allergen for ANPT can be calculated from the dose used in SPT. The concentration producing a 3-mm wheal in SPT or 1/100 of the concentration causing a positive SPT is proposed as the starting dose. For ANPT using a standardized allergen extract, the initial concentration can start from 1:1,000, with 10-fold increases.

Several methods for allergen application are used: paper disks, sprays, or nebulized solutions. When performing ANPT, the parasympathetic reflex mechanism of the contralateral nasal cavity should be considered, and both sides should be tested simultaneously.

The patient should remain seated and hold breath during application, and then exhale immediately to prevent allergens from entering the larynx and lower respiratory tract. Each concentration is applied at 5-minute intervals, and nasal symptoms are evaluated before bilateral nasal resistance is measured. Evaluation criteria are recommended by the Ear, Nose and Throat section of the German Society of Allergy and Clinical Immunology³⁹: 1) At a transnasal pressure of 150 Pa, regardless of whether symptoms occur or total bilateral nasal resistance after stimulation is increased by more than 60% from baseline; 2) The symptom score (**Table 5**) is 4, regardless of total nasal resistance; and 3) total nasal resistance is increased by more than 30% after stimulation at 150 Pa and the patient's symptom score is 3. The allergen concentration is given at 10-fold increases until a positive reaction occurs. The nasal response can be assessed every 15–30 minutes after application, and the possible occurrence of a delayed reaction with new symptoms several hours after the test concludes should also be considered. The patient must be observed for 2 hours after challenge.

False-positive or false-negative results may occur for ANPT. The main causes of false-positive results are as follows: 1) high allergen concentration; infectious or allergic processes in the previous 2–4 weeks; 2) pH of the extract, temperature, and osmotic pressure; and 3) excipients such as phenol, glycerol, or benzalkonium chloride. False-negative ANPT results may be due to the use of contraindicated drugs, nasal surgery in the previous 8 weeks, and atrophic rhinitis.⁴⁰

3. ACPT

ACPT is a diagnostic test used to evaluate the inflammatory response of the conjunctiva after the eyes of a presumed sensitized patient are exposed to allergens.³³ However, despite the fact that it is a safe, simple, rapid tool to assess ocular or other IgE-mediated allergic diseases, it is clearly underused in daily clinical practice.

Indications for ACPT include patients with seasonal keratoconjunctivitis, perennial keratoconjunctivitis, atopic keratoconjunctivitis, and vernal keratoconjunctivitis⁴¹; it can also be used to diagnose occupational allergy such as latex allergy and FA, and to monitor the follow-up of allergen-specific immunotherapy (AIT). ACPT should be performed outside the exposure

Table 5. Scoring criteria for ANPT

Symptoms	0 score	1 score	2 scores
No. of sneezes	0–2	3–5	> 5
Nasal secretion	None	Little (< 1 mL)	Massive (> 1mL)
Extranasal symptoms	None	Palate/eye/ear itchiness	Conjunctivitis, chemosis, urticaria, cough, dyspnoea

period and site to ensure the patient's safety. It should be avoided in subjects with any other ocular disorders, including inflammation/infection of the conjunctiva, cornea, or iris, as well as in cases of severe dry eye syndrome. ACPT is prohibited in patients undergoing ocular surgery in the past 6 months. Additionally, ACPT should be avoided in pregnant and lactating women as well as in patients affected by uncontrolled diseases, particularly uncontrolled asthma, and severe systemic diseases such as autoimmune diseases, heart and vascular diseases, hyperthyroidism, severe liver or renal insufficiencies, and ongoing malignancies.⁴²

When performing ACPT, 20–40 μL of allergen extract should be instilled into the lower outer quadrant of the bulbar conjunctiva of one eye, and the same dose of normal saline as the control into the opposite eye. The recommended initial allergen concentration is 0.1 IU/mL, and the maximum concentration must not exceed 100 IU/mL at 10-fold increases. The interval between the 2 concentrations is 15 minutes.⁴³ The instillation should be stopped immediately once a positive reaction is indicated. Positive criteria (**Table 6**) are based on secondary eye symptoms (*e.g.*, tearing, blepharospasm, and swelling) and signs.⁴⁴ Eye itching is the main positive criterion, usually occurring 3–5 minutes after allergen application, reaching a peak in 10–15 minutes and dissipating after 20 minutes. The itching intensity can be scored on a scale of 0 to 4. Additionally, the visual analogue scale (VAS) has been applied in most clinical studies and can also be used as an alternative method to evaluate the intensity of itching in daily practice. Redness or congestion of eyes is the main sign of conjunctival reaction, appearing 5 minutes after allergen instillation, reaching the peak intensity by 20 minutes and dissipating within 30 minutes. During this process, vascular responses at the ciliary body, sclera, and conjunctival levels must be observed by a physician and, if necessary, can be scored more accurately by slit-lamp examination.

Some issues still exist for ACPT.⁴² The standardization of allergen extracts is required, and the definition of the major allergen content remains an unmet need. The indications for ACPT in extraocular allergy and severe/persistent keratoconjunctivitis remain unclear, warranting further investigations.

4. FPT

FPT is the gold standard for the diagnosis of FA.⁴⁵ The double-blind placebo-controlled food challenge (DBPCFC) is recommended internationally. The dosage is gradually increased on the basis of the protein content in suspected food allergens, and the interval between 2 oral administrations should not be less than 20 minutes. The minimum food dose that induces FA in patients and the maximum food dose without FA are observed.⁴⁶

DBPCFC must be stopped if an allergic reaction is observed or no allergic reactions are observed when the maximum prescribed dose is administered. Immediate reactions usually appear within 2 hours after the last food intake, while atopic dermatitis may appear several hours or days following the challenge. Urticaria and angioedema are the most common

Table 6. Positive criteria for ACPT

Levels	
0	No subjective or visible reaction
1	Itching, reddening, foreign body sensation
2	Stage I and tearing, vasodilation of conjunctiva
3	Stage II and vasodilation and erythema of conjunctiva, blepharospasm
4	Stage III and chemosis, lid swelling

ACPT, allergen conjunctival provocation test.

objective signs, with gastrointestinal, respiratory, or cardiovascular system involvement being also common.³⁴ Food allergens can be identified when allergic reactions occur rapidly after the test and are consistent with the patient's medical history.⁴⁷ Vital signs should be closely monitored during FPT, and professional life-saving equipment and appropriately trained staff should be in place to managed allergic reactions.

In recent years, FPT has been standardized to provide as accurate results as possible. However, several unmet needs have been identified, such as the impact of the maturation stage on the allergenicity of food. Interpretation of the tests might be difficult due to the inconsistency between subjective symptoms described by the patient and the final outcome.

Contraindications into FPT include the following⁴⁶: severe allergic reactions within 1 week; unstable vital signs; uncontrolled asthma; hay fever episodes; acute eczema, atopic dermatitis, urticaria, or unstable period of illness; vaccination within 2 weeks; moderate to severe malnutrition; onset of infectious diseases; chronic underlying diseases, such as unstable angina, congenital heart disease, arrhythmia, chronic lung diseases, cerebrovascular diseases and major organ malformations; chronic digestive diseases; inherited metabolic diseases; mental illness; and pregnancy.

5. DPT

DPT is considered the gold standard for diagnosing DA to NSAIDs, local anesthetics, non-beta-lactams antibiotics, and other drugs for which safer tests are inexistent or not standardized. DPT is also the last diagnostic step for drugs, such as beta-lactam antibiotics, if skin tests and, in some cases, *in vitro* tests yield inconclusive results.⁴⁸

DPT can be used when clinical history strongly supports the diagnosis of DA. If reactions are severe or life-threatening, performing DPT with an alternative drug, rather than the exact drug, is recommended. When a surrogate is not available and the drug is absolutely needed to treat a life-threatening illness, DPT can also be considered.⁴⁹ DPT should be carried out ideally with the culprit drug. Theoretically, the route of administration should be the same as the one evoking the reaction, but international guidelines favor the oral route. Adjustments for the dose, dose interval, number of doses, and total dose given are made according to the drug involved, the initial reaction, patient's conditions, and future needs for drug administration. For immediate reactions, a 30- to 60-minute dose interval is usually appropriate. For a late response, this observational period may take 24 hours or longer.⁵⁰

Although DPT is considered the gold standard for DA diagnosis, unrecognized confounders (*e.g.*, infections, underlying disease and its control, concomitant medications, exercise, and food intake) might influence the reaction. Additionally, vague complaints and nonobjective signs are difficult to ascertain. Therefore, DPT is not 100% sensitive and specific. However, the negative predictive value is high for the most frequently involved drugs such as beta-lactams (94%–98%)⁵¹ and NSAIDs (96%).⁵²

A negative test does not guarantee tolerance to the drug in the future because it rather excludes DA only at the time when DPT was carried out.

DPT should be carried out under strict surveillance and by trained staff in an appropriate setting equipped to deal with anaphylaxis or other severe reactions. DPT should be carried out at least 1 month after the reported reaction to avoid false negatives.

Appropriate preparations should be made before the challenge test: collection of medical history, comprehensive evaluation of the challenge site, assessment of any factors that may affect the test results, and performing skin tests and/or serological tests to fully evaluate the need for challenge testing.

DPT may induce severe allergic reactions and cannot be used as a routine clinical test. It should be performed only when necessary.

Patch test

Patch test is currently the simplest and most reliable method to diagnose contact dermatitis (CD). It is the gold standard for CD diagnosis. In patients with a history of more than 3 months or recurrent CD, patch test is recommended to determine the effects of contact allergens in the environment. There are 2 possibilities for positive patch tests: irritant and allergic reactions. CD can be divided into irritant contact dermatitis (ICD) and allergic contact dermatitis. An immune response or sensitization is not involved in ICD. The degree of irritation is mainly related to the irritant concentration and time of contact with the skin. In patch tests, it is generally considered that erythema caused by irritant reactions disappears quickly after the test substance is removed, while allergic reactions are manifested as symptoms such as infiltrative erythema and papules. A positive response often occurs more than 72 hours or longer after the patch test. However, it is difficult to accurately distinguish irritant reactions from allergic reactions by a positive result of a single patch test, particularly in the case of mild reactions.⁵³

The patch test system comprises 3 parts: test chambers, allergens, and the tape. Commercially available test systems are generally integrated systems. They can be applied directly if the cover is peeled off according to the instructions for use. The allergen dose per unit area of skin contact is accurately quantified by each chamber and is independent of each other. The allergen panel is designed according to the distribution characteristics of different countries or regions. The test should be performed on the back, with the upper back being the best site. Affected by factors such as the structure and concentration of allergens, and the sensitivity of the test subject, there is no strict standard for how long the patch is applied and the optimal time to observe responses. Time to read results varies from 2 to 7 days, depending on manufacturers. The test results are measured by morphological criteria (**Table 7**) recommended by the International Contact Dermatitis Research Group.⁵⁴

The allergic reaction is generally palpable raised erythema. In severe cases, there may be vesicles or coalescing vesicles, with obscure boundary. The rash can extend outside the test chamber even like a thin red line spreading along the lymphatic vessels, and itching is obvious. The rash may still worsen after removing the test substance (allergens) and then gradually vanish within several days. It must be distinguished from irritant reactions during

Table 7. Reporting of patch test results, according to the ICDRG

Results	Meaning	Responses
–	Negative	Normal reaction
±	Doubtful	Only mild erythema
+	Moderate reaction	Erythema, infiltration and possible papules
++	Strong reaction	Erythema, infiltration, papules and vesicles
+++	Very strong reaction	Intense erythema, infiltration and vesicles or coalescing vesicles
IR	Irritant reaction	Local wrinkled paper-like reaction

ICDRG, International Contact Dermatitis Research Group.

interpretation. The latter shows a local wrinkled paper-like reaction, which can also behave exactly like an allergic reaction. Furthermore, patch tests are not recommended during the acute attack of dermatitis to prevent generalized dermatitis. Medications that may affect the test, such as glucocorticoids, immunosuppressive agents as well as traditional Chinese medicines and extracts with immunosuppressive effects such as *Tripterygium Wilfordii* preparation, should be stopped. Antihistamines theoretically have no impact on this test, which is controversial. Patch tests should not be performed within 4 weeks after phototherapy with ultraviolet exposure, sun exposure, or other radiographic exposure due to their inhibitory effects. It is important not to wash or scratch the application area or to perform vigorous exercise during the test. Sweating and sun exposure should also be reduced. If the skin reaction at the test site is strong, particularly pain or a burning sensation, the test substance should be immediately removed.⁵⁵

In vitro allergen tests

In vitro allergy diagnostics comprises serological and cytological tests. The former mainly detects tIgE and sIgE to allergens and their components. Blood cells are used as test samples in cytological tests, and the most common application is BAT.

tIgE test

IgE is an important immune molecule generated during a type I hypersensitivity reaction. Monitoring of increases in tIgE levels cannot be used to diagnose allergic disease because it is only predictive of a type I hypersensitivity reaction. Moreover, other diseases can lead to significantly increased tIgE levels, such as various autoimmune diseases and immune system deficiencies as well as parasitic and microbial infections.^{56,57} Furthermore, patients with allergic diseases may show normal tIgE levels. Consequently, the clinical significance of relying solely on tIgE test results is limited. In clinical practice, results must be combined with the patient's medical history and clinical symptoms to provide a comprehensive evaluation.

The serum tIgE level is a valuable marker for the diagnosis and follow-up of ABPA.⁵⁸ One of the essential criteria for the diagnosis of ABPA is a tIgE level of >1,000 IU/mL, and the sensitivity and specificity of this cutoff value are approximately 92% and 40%, respectively.^{59,60} The serum tIgE level also plays an important role in the monitoring of ABPA patients. An elevated level of tIgE during the follow-up period may indicate degeneration of a patient's condition.⁶⁰

The distribution of the tIgE level in human serum is skewed, making it difficult to define the reference interval. Longitudinal studies have shown that the tIgE level gradually increases with age after birth and reaches the adult level at the age of 12 years, that a level above 333 IU/mL is considered abnormally elevated in adolescents.⁶¹ The reference intervals of the serum tIgE level in children are as follows: < 12 IU/mL for those younger than 2 years, < 33 IU/mL for those 2 to 4 years, and < 85 IU/mL for those 4 to 15 years; the reference interval of the serum tIgE level for adults is approximately < 125 IU/mL.⁶² A higher serum tIgE level in infants and children indicates a higher risk of developing allergic disease in adulthood.⁶¹

sIgE test

1. sIgE to allergens

The sIgE test of allergens plays a key role in the *in vitro* diagnosis of allergic diseases. The higher the sIgE level is, the stronger its correlation with allergic disease is.⁶³ In recent years, with advances in immune-labelling technology, *in vitro* diagnostic techniques for allergens

Table 8. Comparison of different sIgE detection methods

Detection principle	Detection method	Detection type	Advantages	Disadvantages
Fluorescence immunoassay using cellulose	Immune fluorescence assay	Quantitative	High sensitivity and specificity; as the “gold standard” of allergen detection <i>in vitro</i> in the international arena; fully automated.	High cost of single allergen testing; high volume of sample required (40 µL/test); large volume of the instrument; strict daily maintenance requirements for operators; high instrument and reagent price.
96-well microplate	Enzyme-linked immunosorbent assay	Quantitative/semi-quantitative	Manual, semi-automatic and fully automatic operation; flexible combination of IgE and IgG detection.	Strict temperature requirements for reagents.
Microfluidic biochips	Chemiluminescence immunoassay	Quantitative/semi-quantitative	High sensitivity; easy to operate; lower cost and more stable reagents.	Lack of fully automatic operation options.
Microarray	Microarray enzyme-linked immunoassay	Semi-quantitative	Automation of analytical processes; reporting results for over 40 common allergens in 35 min at a serum requirement of 100 µL, making it suitable for both outpatient and primary care settings.	Semi-quantitative results, lacking in accuracy.
Cellulose nitrate membranes	Western blotting	Semi-quantitative	Automated operation and result interpretation; screening of maximum allergens with minimum sample.	Semi-quantitative results, lacking in accuracy.
Colloidal gold	Colloidal gold method	Qualitative	Easy to operate; simultaneous detection of tIgE and multiple independent sIgE; no reagents and the method used to interpret the sample preparation; results can be read with the naked eye.	Sensitivity varies according to the quality of the results.

IgE, immunoglobulin E; IgG, immunoglobulin G; sIgE, specific immunoglobulin E; tIgE, total immunoglobulin E.

have been developed.⁶⁴ Different domestic and imported reagents have also emerged in China for *in vitro* allergen detection. The primary detection methods depending on the coated solid phase carriers are shown in **Table 8**.

There has been substantial movement in allergy diagnostics over the past 10 years. In terms of methodology, improved technologies appear to offer solutions that could make testing easier to manage, reduce the sample requirements, and provide more efficient detection of multiple allergens simultaneously. It remains to be determined whether some or all of these technologies emerge as viable offerings in a central laboratory rather than simply research tools that can be used for allergy diagnostics.

The interpretation of allergen-sIgE results is shown in **Table 9**. The sIgE concentration objectively reflects sensitization status, and major allergen can be identified by a positive result. Additionally, quantitative testing provides guidance and monitoring for AIT; however, the grade of sIgE result is not necessarily correlated with the severity of disease. Furthermore, a positive sIgE does not always align with clinical symptoms.

2. sIgE to allergen components

Perhaps the most important advancement in allergy diagnostics over the past 20 years is the use of individual proteins (components) from an extract to more definitively address allergic disease. Researchers have spent substantial time identifying individual allergenic

Table 9. Interpretation of allergen-sIgE results

sIgE concentration (IU/mL)	Grade	sIgE level	Clinical implication
< 0.35	0	Negative or undetectable	Non sensitisation
≥ 0.35 and < 0.70	1	Low	Possible or mild sensitisation
≥ 0.70 and < 3.50	2	Increased	Mild sensitisation
≥ 3.50 and < 17.50	3	Significantly increased	Moderate sensitisation
≥ 17.50 and < 50.00	4	High	Moderate to severe sensitisation
≥ 50.00 and < 100.00	5	Very high	Severe sensitisation
≥ 100.00	6	Extremely high	Extremely severe sensitisation

sIgE, specific immunoglobulin E.

species within an extract, understanding reactivities of patient populations to those proteins, and thereby improving the fidelity of *in vitro* testing. To date, more than 900 allergenic components have been identified and are available in various forms for allergy diagnostics.

A defined nomenclature has been established for the components of a given allergen; the designation utilizes the first 3 letters of the genus (Latin name) for that allergen accompanied by a space and then the first or 2 letters of the species, joined by a space, followed by an Arabic number. The numbers are assigned to the allergens in the order of their identification, or based on their codes in the protein family to which they belong.⁶⁵ For example, the designation for the major allergenic lipid transfer protein (LTP) from peach (*Prunus persica*) is Pru p 3. Allergens with different amino acid sequences within a species continue to be divided into isoallergens (*e.g.*, Pru p 1.01) and variants (*e.g.*, Pru p 1.0101) according to the degree of similarity.

The WHO and International Union of Immunological Societies Allergen Nomenclature Subcommittee, founded in 1984, is responsible for maintaining a systematic nomenclature for allergens and has 930 allergen components registered in the database (<http://allergen.org/>). Chinese researchers have identified and submitted numerous new allergens, including mite components (*Dermatophagoides pteronyssinus* [Der p] 24 and *Dermatophagoides farinae* [Der f] 24), artemisia components (Art ar 2 and Art an 7), crab component (Eri s 2), and cockroach components (Per a 11 and Per a 12).

Although hundreds of allergens have been identified according to their species, they are classified into more than 20 families according to their protein structures, an important theoretical basis for allergic cross-reactivity.⁶⁶ Recently, the COMPARE database (Comprehensive Protein Allergen Resource, <https://comparedatabase.org/>) was launched by the US Health and Environmental Sciences Institute with the support of an international team of immunologists in allergy, to provide scientific and accurate information on the identification, sequencing and naming of allergenic proteins. The COMPARE database allows users to search for the individual sequences of allergen components and predict levels of allergenicity for new allergens.

In early 2016, the EAACI published the Molecular Allergy User's Guide,⁶⁷ which offers a comprehensive overview of molecular allergy from basic research to the most recent practices in the clinical laboratory. This guide outlined that the use of recombinant or purified native components in allergy diagnostics has led to the emergence of component-resolved diagnostics (CRD) in many international clinical laboratories.^{68,69} Although traditional allergen extracts usually contain low or very low concentrations of allergen components, CRD can use higher concentrations of purified material that improves the accuracy and comprehensive value of sIgE. To that end, Chinese investigators have made important progress utilizing component allergens in the diagnosis of allergy to dust mites, birch pollen, artemisia pollen, peach, and milk.

CRD has been extremely helpful in identifying cross-reactivity among allergens and major allergenic proteins; this has led to a better understanding of multiple factors involved in allergic hypersensitivity and has helped improve the diagnostic accuracy. For example, *Der p* 1 and *Der p* 2, along with *Der p* 23, are the most frequently recognized allergens in house dust mites (HDM). *Der p* 10 is cross-reactive with shrimp and crab allergens.⁷⁰ LTPs are present in peach (Pru p 3), artemisia (Art v 3), peanut (Ara h 9), and Chinese chestnut (Cor a 9); the common cross-reactivity shared among these proteins is associated with severe allergic reactions.^{71,72}

CRD allows differentiation between genuine positive sIgE results and cross-reactivity. For example, Pru p 1 and Pru p 3, major allergens of peach, cross-react with birch pollen Bet v 1 and artemisia pollen Art v 3, respectively.^{72,73} Moreover, CRD can be utilized to predict the potential risk of disease; patients who are sensitive to multiple components in artemisia demonstrate a significant risk for asthma.⁷⁴ Furthermore, accuracy in diagnosis of allergy to milk components is improved using CRD.⁷⁵

Allergen components can also be used to qualify patients for AIT, thereby improving the efficacy of AIT. A recent study has demonstrated that in the use of *Der p* extracts in AIT, patients sensitized to *Der p* 1 and *Der p* 2 are more effective than those sensitized to other HDM components.⁷⁶ This is likely that HDM extracts used for AIT are generally standardized only for *Der p* 1 and *Der p* 2, with a low content of other allergen components; the components *Der p* 1 and *Der p* 2 are strongly immunogenic, while other components in the allergen extract are less immunogenic and do not induce as substantive IgG responses. Therefore, the authors believe that CRD can assist doctors in screening patients most suitable for AIT using HDM extracts to improve clinical efficacy.

The sIgE of allergen components can be determined using a singleplex (one assay per sample) or multiplex (multiple assays per sample) measurement platform. A singleplex platform allows physicians to select allergen components necessary for an accurate diagnosis defined by the clinical history of the patient. The multiplex approach allows for characterization of the IgE response against a broad array of preselected allergens independently of the clinical history.

1) Singleplex method for measurement of sIgE to allergenic components

The singleplex method measures sIgE to a single allergen (extract or component) in an individual reaction vessel. In general, the sensitivity of the singleplex method is superior to that of a chip coated with multiple allergen components in arrays. However, when there are many allergen components involved (as in pollen and dust mites), the cost per patient could be a deterrent.⁷⁷

Over the past decade, China has made multiple contributions to clinical studies using the platform developed by Phadia (an allergen detection system from Uppsala, Sweden); these studies have exerted a broad influence on allergy diagnostics both domestically and internationally, and have increased a general understanding of CRD in China.

2) Multiplex method for the measurement of sIgE to allergenic components

The multiplex approach has been pioneered in allergy diagnostics through a microarray chip. This technology measures multiple allergens (primarily components) simultaneously in a single biochip. In general, the methodology is designed more toward research laboratories but uses a relatively small sample size. The use of array-based multiplex methods can help identify cross-sensitization species as well as potentially high-risk allergens.

Currently, there are 3 multiplex platforms for allergen components abroad:

- First generation (year 2000): Immuno Solid-phase Allergen Chip, sIgE capable of 112 allergens and their components
- Second generation (year 2011): MeDALL-chip, sIgE capable of 151 allergens and their components
- Third generation (year 2017): ALEX platform, sIgE and tIgE capable of 282 allergens and their components as well as cross-reactive carbohydrate determinant (CCD) inhibition

The multiplex method is suitable for patients sensitized to both food and inhaled allergens. It is also recommended for use in population-based epidemiological studies assessing the spectrum of specific allergen components.

3) Singleplex or multiplex platforms applications in a clinical setting

Recombinant or naturally purified allergen components can be used in CRD, and sIgE can be measured using singleplex or multiplex platforms. In general, for complex cases sensitized to multiple allergens and patients with severe allergic reactions, the multiplex CRD method should be performed. The multiplex platform enables simultaneous IgE test of more than 200 allergen components with a very small volume of serum (100 μ L), but with insufficient sensitivity compared to singleplex assays. Additionally, multiplex assays utilizing cellulose with different CCDs as a solid-phase allergen carrier may cause false-positive test results in patients with a high sIgE of CCD. These false-positive results occur due to anti-cellulose IgE present in the patient's sample; a CCD inhibitor procedure should be required to resolve these problems.⁷⁸

sIgG4 test

IgG is the most abundant type of antibody in the serum, accounting for about 75% of serum immunoglobulins. IgG includes 4 subtypes: IgG1, IgG2, IgG3, and IgG4. Among them, IgG4 has the lowest content, about 0.5 mg/mL, and the inter-individual difference fluctuates between 10 μ g/mL and 1.4 mg/mL.

AIT is currently the only etiological treatment that can change the natural course of allergic disease. During AIT for people who are allergic to insect venom, decreases in the sIgE levels are often accompanied by corresponding increases in sIgG4 levels.^{79,80} The sIgG4 induced by AIT not only serves as “blocking” antibodies,^{81,82} but also prevents the allergen-induced release of histamine.⁸³ However, the determination of sIgG4 can only reflect the patient's immune response to AIT, and its increase is not equal to the success of AIT.⁸⁴

Importantly, sIgG and sIgG4 of food allergens are not recommended to screen and diagnose FA or food intolerance,^{45,85-87} because they can be detected in both healthy and sick individuals.⁸⁸ Thus, positive results of sIgG only indicate the body's normal immune response.

BAT

Basophils are a type of white blood cell (WBC) present in peripheral blood. These cells are extremely rare, constituting about 0.2% of the total number of WBCs in blood. Basophils are important effector cells of type I hypersensitivity.⁸⁹ BAT can be performed when the patient's medical history is inconsistent with the sIgE or skin test results, or the patient has experienced a systemic allergic reaction during the skin test.⁹⁰ BAT can be used to identify food allergens and allergenic cross-sensitization as well as monitor the effectiveness of AIT and anti-IgE therapy. The principle of BAT is to stimulate the patient's peripheral blood with suspected allergen extract; the basophils are then degranulated after activation by the allergen of interest through IgE-/non-IgE-dependent pathways. There are 2 ways to monitor basophils degranulation: one is to detect the expression of basophilic surface membrane markers such as CD63/CD203c by flow cytometry, with the degree of basophils estimated,⁹¹ while the other is to measure inflammatory mediators such as histamine based on enzyme-linked immunosorbent assay. These 2 methods presume the specific allergen sensitized the patient through the up-regulated expression of activation markers or the release level of mediators like histamine.

BAT is regarded as an *in vitro* challenge test based on cell function. This method is superior over *in vivo* challenge test in terms of wide applicability, good reproducibility, safety, and time saving for the detection of allergen. Most importantly, it can protect patients from severe allergic reactions caused by *in vivo* tests. The results of BAT more accurately reflect the patient's current sensitivity to allergens in contrast to serum sIgE; compared to the sIgE test, BAT has a wider detection range and can even detect some small molecule drugs.⁹²

All diagnostic tests have advantages and limitations (**Table 10**), and physicians must carefully choose a diagnostic test according to the patient and medical conditions.

COMMON AEROALLERGENS AND THEIR DISTRIBUTION IN CHINA

Common aeroallergens in China

Aeroallergens are a series of airborne allergens that can cause allergic disorders in sensitized individuals. Major aeroallergens are derived from dust mites, pollens, cockroaches, animal dander, and fungi. Presently, various screening panels, ranging from several to dozens of allergens, are used by different hospitals in different provinces and cities of China. The regional distribution of allergens varies according to climate differences. For example, dust mites and fungal allergens may be common in south China, while pollens and dust mites are primary allergens in north China. Therefore, allergens available for a given region should be precisely used because the nonselective use of generic allergens for screening increases the economic burden on patients and society. Additionally, because an indefinite correlation between the results and clinical symptoms, blind screening for many allergens will most likely lead to misdiagnosis by physicians.

Screening panels of aeroallergens

It is important to develop a screening panel based on the spectrum of local epidemic allergens to improve the diagnosis and treatment of region-specific allergic diseases. In Europe, according to the Global Allergy and Asthma European Network (GA²LEN) study, 4 to

Table 10. Strengths and limitations of diagnostic or observational tests

Diagnostic tests	Indications	Strengths	Limitations
Skin test	Type I hypersensitivity	Rapid diagnosis and high specificity	Can be affected by skin condition and medication, has the risk of inducing allergic reaction.
Provocation test	Allergic disease	“Gold standard” for the diagnosis of allergic diseases	Risk of inducing anaphylaxis.
Patch test	Contact dermatitis	“Gold standard” for diagnosis of contact dermatitis	Can be affected by medication (corticosteroids, immunosuppressants), and can cause a certain degree of local skin lesion.
tIgE test	Allergic disease	Can reflect atopic constitution to a certain extent, necessary condition for the diagnosis of ABPA	In addition to allergic disease, various other diseases can lead to increased tIgE.
sIgE test	IgE mediated allergic disease	High sensitivity to diagnosis, not affected by drugs	The detection of crude extract of allergen could not distinguish the cross reaction.
Component-resolved diagnostics	IgE mediated allergic disease	Accurate diagnosis helps to distinguish cross-sensitisation and co-sensitisation	The detection cost is high, and little reagent is available.
Specific IgG4 test	Allergen specific immunotherapy	Serves as a monitored marker for the tolerance inducing effect of allergen specific immunotherapy	It cannot be used for the diagnosis of allergic diseases.
Basophil activation test	Allergic disease	Wide applicability, good repeatability and high safety, not affected by medication	High cost, currently mainly used in scientific research, clinical diagnosis is not widely used.

IgE, immunoglobulin E; IgG, immunoglobulin G; tIgE, total immunoglobulin E; sIgE, specific immunoglobulin E; ABPA, allergic bronchopulmonary aspergillosis.

Table 11. Region-specific common allergens and their sensitisation rates in China

Regions	Top 8 allergens in different regions and their sensitisation rates							
Mainland China	<i>Der f</i> 47.2%	<i>Der p</i> 41.4%	<i>Blatella</i> 33.3%	Mugwort 28.6%	Dandelion 24.9%	Ragweed 23.5%	Hazel 21.7%	Birch 20.6%
Northeast China	<i>Der f</i> 43.1%	<i>Der p</i> 38.8%	Mugwort 33.6%	Ragweed 29.0%	Dandelion 28.3%	<i>Blatella</i> 23.5%	Hazel 19.9%	Birch 18.9%
Northwest China	Mugwort 58.2%	Ragweed 47.1%	Dandelion 45.3%	Goosefoot 38.3%	Locust 29.3%	Hazel 28.7%	Plantain 27.4%	Grass pollen 26.7%
Central China	<i>Der f</i> 47.3%	<i>Der p</i> 41.7%	<i>Blatella</i> 34%	Mugwort 27.1%	Dandelion 24.1%	Ragweed 22.2%	Hazel 22%	Birch 21%
South China	<i>Der f</i> 69.2%	<i>Der p</i> 61.4%	<i>Blatella</i> 39.4%	Mugwort 14.9%	Ragweed 14.3%	Dandelion 11.7%	Locust 7.8%	Hazel 7.7%

Der f, Dermatophagoides farinae; *Der p*, Dermatophagoides pteronyssinus.

13 of a set of 18 allergens were required to identify 100% of sensitized subjects.^{19,93} In Korea, Lee *et al.*⁹⁴ found that, in a cohort of 5,032 AR patients, only 5 to 7 allergens in a combination containing 55 SPT allergens are sufficient to confirm 93% to 95% of these patients. Recently, 7,148 subjects with self-reported AR in a study from China were subjected to SPT, and the results showed that 8 of the 20 allergens (*Der p*, *Der f*, artemisia, *Blatella*, hazel, goosefoot, *Penicillium notatum*, and animal dander) allowed the identification > 96% of sensitized subjects in central China.⁹⁵ The top 8 allergens in different regions are shown in **Table 11**.

The prevalence of various aeroallergens in China varies according to geographical areas, climates, and environmental and economic factors. Numerous epidemiological surveys from China showed that dust mites, particularly *Der p* and *Der f*, are the major aeroallergens that cause respiratory allergic diseases (*e.g.*, asthma and rhinitis) in the central, eastern, and western regions of China as well as some northern cities.⁹⁶⁻¹⁰⁰ Similarly, another allergen SPT study of AR and/or asthmatic patients from 17 cities located in northern, eastern, southwestern and southern coastal areas of China also showed that *Der f* (59.0%) and *Der p* (57.6%) are primary sensitizers.⁸ However, pollen allergens are predominant in northwestern China.¹⁰¹⁻¹⁰⁴ The distribution of pollen allergen is highly regional and seasonal, which is affected by climatic conditions and time. Therefore, the prevalent pollen allergens of trees and grasses in different regions of China are also different.^{105,106} Hay fever in spring is mainly caused by tree pollens, while the chief culprit in summer and autumn are herbaceous plants.¹⁰⁷ Based on the above study findings, the following allergens are proposed to be included in a skin test or sIgE test panel: *Der p*, *Der f*, *Blattella germanica*, *Blomia tropicalis*, dandelion, *Bermuda grass*, and birch for South China; artemisia, ragweed, *Humulus scandens*, *Der p*, *Der f*, goosefoot, *Blattella germanica*, and *Bermuda grass* for Northwest China; *Der p*, *Der f*, artemisia, *Humulus scandens*, *Blatella*, *Bermuda grass*, goosefoot, and dandelion for Northeast China.

Furthermore, animal dander (*e.g.*, cat and dog) and fungi (*e.g.*, *Alternaria*, *Penicillium*, *Aspergillus fumigatus*, and *Clostridium*) can be added according to the patient's history of pet contact and living environment. A screening panel that includes reasonable aeroallergens is the most cost-effective way to detect allergens.

CLINICAL RESPONSE INDICATORS OF AIT

AIT is an effective treatment for AR and/or allergic asthma, which can improve symptoms and enable patients to achieve long-term clinical benefits after treatment.

The efficacy of AIT is evaluated by 2 indicators: clinical symptoms and medication scores on allergen exposure. There are currently no simple and practical biomarkers to assess the efficacy of AIT and to monitor the prognosis. The ratio of sIgE to tIgE (sIgE/tIgE ratio) was demonstrated to be as a predictive marker in a group of patients who had received AIT for

4 years against grass pollen or HDM.¹⁰⁸ Additionally, the clinical response was evaluated using the VAS. The authors found that sIgE/tIgE ratio greater than 16.2% correlated with an effective clinical response to AIT, with a sensitivity of 97.2% and a specificity of 88.1%.

The effect of AIT correlates with the allergen sIgG antibody responses, particularly sIgG4. The sIgG4 concentrations showed a 10- to 100-fold increase after the patients received AIT.¹⁰⁹ It is currently believed that IgG4 and sIgE competitively bind allergens and inhibit formation of antigen-antibody complexes on mast cells, basophils, and other sIgE receptor-expressing cells.^{110,111} However, according to the symptoms and medication scores, the change in the IgG4 level was found to be not well correlated with the efficacy of AIT. A previous study found that the levels of *Der p*-sIgG4 in patients receiving HDM immunotherapy showed a significant increase from week 12 (the maintenance phase).⁸⁴ A close relationship was demonstrated between *Der p*-sIgG4 and clinical efficacy during the maintenance phase rather than the up-dosing phase of AIT. Immunological tolerance can be induced with AIT when the maintenance phase is achieved. Additionally, in several studies of sublingual-swallow immunotherapy, the ratio of sIgE to sIgG4 decreased and was related to skin reactivity¹¹²; however, subsequent studies have not reported consistent results.¹¹³ Therefore, whether the sIgG4 and sIgE/sIgG4 ratio can be used to evaluate the efficacy of AIT is inconclusive.

In summary, the sIgE/tIgE ratio is predictive of the clinical efficacy of AIT. Quantification of the sIgG4 level in the maintenance phase can be used to evaluate immunological tolerance and to monitor the efficacy of AIT. However, these findings are inconclusive, so further studies are warranted.

CONCLUSION AND PERSPECTIVES

The detection of allergens is critical for the prevention and treatment of allergic disease. Currently, technology available in China is insufficient; however, with the introduction of new microparticle- and array-based systems, many of the diagnostic gaps observed in China can soon be closed. This consensus summarizes available *in vivo* and *in vitro* diagnostic methods for allergens to guide allergists and laboratory technicians, intending to improve the diagnostic level and to guide the treatment of allergic disease for the benefit of patients.

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