Section C

ALLERGY DIAGNOSIS

* Skin tests
* Nasal and bronchial provocation tests
* Food provocation tests
* In vivo diagnosis of NSAID hypersensitivity (Aspirin provocation tests)
* Drug provocation tests

* The allergen challenge chamber
* Allergen-specific IgE
* Molecules and component-resolved diagnosis
* Cellular allergy testing
* Biomarkers for allergy diagnosis and treatment
Skin tests are an important diagnostic tool in the work up of many allergic diseases (Table 1). Skin tests are widely used for the diagnosis of inhalant allergies, but also allergies to foods, venom, occupational agents and drugs can be investigated by skin tests.

**TYPES OF SKIN TESTS AND REQUIREMENTS**

Skin prick tests (SPTs) and intradermal tests are still the cornerstone of the diagnosis of IgE-mediated (type I) allergy. Especially SPTs are widely used (Figure 1). As they are usually easy to perform, cheap and allow a fast reading, they are an ideal tool for bedside diagnosis. Intradermal also named intracutaneous testing (Figure 2) is more demanding, but allows by titration of various allergen concentrations a very accurate rating of the sensitization level.

Epicutaneous or patch tests are used in patients with suspected type IV allergies for contact dermatitis or delayed type of drug hypersensitivity. Due to its potentially scar-inducing effect skin testing by scratching is limited to some special aspects of drug or mostly occupational allergens. The atopy patch tests (APT) may help to find out causes of exacerbation in atopic dermatitis or Eosinophilic Esophagitis, but its value is still controversial. Performing skin tests needs a specific training, especially for intradermal and epicutaneous tests with nonstandardized allergen material.

Contraindications such as the use of some drugs, pregnancy or other conditions precluding some types of allergy testing should be evaluated. Also the skin condition of the patient must allow testing in the skin. Patients have to abstain from medications such as antihistaminics that could mask a positive type I reactions; on the other hand conditions such as dermographism/pressure urticaria able to provoke false positive skin reactions must be considered. Using controls such as histamine for positive and saline as negative controls are crucial for SPT/intradermal testing.

**SKIN TESTS FOR INHALANT ALLERGENS**

SPT are the classical diagnostic tool get prompt information about sensitizations against inhalant allergens such as pollen, house dust mites, pets and, to a lesser extent moulds. As they are usually easy to perform, cheap and allow a fast reading, thus an ideal tool for bedside diagnosis. Recommendations for standard series of SPT solutions are available for many geographic and climatic regions. Skin prick tests can also be used in tropical areas and in countries with limited resources, where allergy is booming. However, local allergens may not necessarily be identified or available and can therefore be missed for skin testing. For example, it is crucial to include allergens such as *Blomia tropicalis* in the skin test battery of tropical countries.
### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Skin Prick Test</th>
<th>Intradermal Tests</th>
<th>Epicutaneous (Patch) Tests</th>
<th>Atopy Patch Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhalant allergies</strong></td>
<td>+++ Standard</td>
<td>Rarely needed, few available allergens</td>
<td>n.a.</td>
<td>Some forms of atopic dermatitis¹</td>
</tr>
<tr>
<td><strong>Food allergy</strong></td>
<td>++ With commercially available extracts and fresh food</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Eosinophilic esophagitis? Some forms of atopic dermatitis²</td>
</tr>
<tr>
<td><strong>Venom allergy</strong></td>
<td>+ (reduced sensitivity)</td>
<td>+++ (Titration)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Drug hypersensitivity</strong></td>
<td>+ to ++ (with all soluble drugs; Titration)</td>
<td>+++</td>
<td>(++) In delayed drug hypersensitivity³</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Contact allergens</strong></td>
<td>(+) (in case of protein contact dermatitis)</td>
<td>n.a.</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

¹ If Atopic Dermatitis seems to exacerbated by the presence of inhalant allergens
² If Atopic Dermatitis seems to exacerbated by the presence of food allergens
³ Especially if cutaneous manifestations of delayed type (eczema, maculo-papular rash) occur

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**Figure 1** Skin prick test with a) inhalant allergens and b) fresh food.
SKIN TESTS FOR FOOD ALLERGY
For food allergy mostly SPT is used, done with commercially available food allergen extracts but also with fresh food (Prick-to-Prick technique, Figure 1b). Testing with fresh food enables a fast detection of sensitization also to foods individually provided by the patient or only locally available. As skin testing does not discriminate between sensitization and clinical relevant food allergy, additional tools such as food allergen oral provocation tests, elimination diets and more recently component-resolved in vitro diagnosis are indicated. Intradermal testing with food allergens is not recommended.

SKIN TESTS FOR HYMENOPTERA VENOM ALLERGY
Skin testing for hymenoptera venom allergy is a very reliable and useful diagnostic tool. While SPT with venoms has limited sensitivity, intradermal testing with increasing concentrations allows the detection of the sensitization threshold, with high sensitivity and specificity. Concentrations starting at 0.00001 μg/ml with a ten-fold increase in doses at each step till 1.0 μg/ml are used. Simultaneous testing with two hymenoptera venoms seems to be safe and allows fast bedside diagnosis.

SKIN TESTS IN DRUG HYPERSENSITIVITY
Skin tests are a very useful but also delicate tool to investigate drug hypersensitivity. Especially for intradermal testing of soluble drugs, the ideal concentrations need to be evaluated. In a recent position paper of the ENDA/EAACI Drug Allergy Interest Group drug concentration for skin testing aiming to achieve a specificity of at least 95% have been postulated. Currently such drug concentration can be recommended for beta-lactam antibiotics, perioperative drugs, heparins, platinum salts and contrast media. For many other drugs, there is still insufficient evidence to define an appropriate drug concentration.

KEY REFERENCES
INTRODUCTION
Epidemiological studies have shown that inhaled allergens are an important cause of allergic rhinitis (AR) and asthma. Allergen inhalation challenge mimics the natural situation and is useful for understanding the mechanisms of allergic airway inflammation and airway hyperresponsiveness (AHR). Moreover, provocation tests play an important role in the diagnosis of asthma, and to a lesser extent also in AR. Via a bronchial provocation test (BPT), AHR can be measured by challenging the airways with a variety of physical and inhaled chemical stimuli resulting in airway constriction (Figure 1). The airway narrowing is measured by changes in FEV1 after gradually increasing the dose of provoking agent.

A nasal provocation test (NPT) is used in case of a typical history of AR and a negative allergy test (Figure 2). It is of importance to differentiate AR from non-allergic rhinitis since management may be different. A positive test result is defined by the presence of symptoms typical for AR plus objective parameters, such as decreased upper airway patency (measured with acoustic rhinometry or anterior rhinomanometry) or nasal secretion of inflammatory mediators. Patients can have immediate or dual responses.

METHODS
Different methods for NPT and measurement of nasal responses are used. Medication that may interfere with the results should be stopped prior to the test and contra-indications should be ruled out. If available, it is advised to follow a validated and standardized protocol.

Inhaled allergen challenge starts early in the morning in order to measure a late asthmatic response. Ideally, patients are observed for a longer period. It is strongly recommended to perform BPT in a clinical setting. In daily clinical practice for AHR diagnosis, the preferred agents are histamine, methacholine and mannitol. These agents give a bronchoconstrive response that is less prolonged and quickly reversible with bronchodilators and inhaled corticosteroids. Diagnosis of occupational asthma and rhinitis may require specific inhalation tests.

LIMITATIONS
Inhaled allergen provocation tests are safe in a dedicated setting with experienced staff. Several objective and validated parameters to measure response are available for BPT, but not for NPT. Allergen application may produce nonspecific reactions. A limited number of standardized allergen extracts are commercially available. Allergen challenge can result in a transient increase in symptoms and medication use.
**TABLE 1**

**Indications for NPT or BPT**

- To confirm a diagnosis of occupational allergic rhinitis or asthma.
- Discrepancy between history and routine diagnostic procedures.
- To confirm a diagnosis in a patient who has difficulty accepting the consequences of disease, such as avoiding pets or changing jobs.
- To study pathophysiological mechanisms and pharmacological efficacy of medication.

**TABLE 2**

**Contra-indications for NPT or BPT**

- Advanced nasal polyposis (NPT)
- A recent history of nasal surgery (NPT)
- Respiratory tract infection in the last 2 weeks.
- Instable asthma (BPT)
- FEV1 < 70% of predicted (BPT)
- Pregnancy
- Recent vaccination (< 1 week)
- Use of systemic Beta-blockers

Figure 1 Mechanism of action in bronchial provocation tests. (Reproduced with permission from O’Byrne PM, Gauvreau GM, Brannan JD. Provoked models of asthma: what have we learnt? Clin Exp Allergy 2009;39:181-92, with permission from Willey Blackwell.)
SAFETY AND SIDE EFFECTS
NPT and BPT are safe procedures. Generalized reactions are rare after BPT, but asthma exacerbations may occur. After allergen challenge the magnitude of the bronchoconstriction may be more difficult to control than with direct challenges, such as histamine or methacholine. Moreover, the induced bronchoconstriction is usually prolonged due to the release of proinflammatory mediators. Therefore, it is necessary that during an inhaled allergen provocation a doctor is present, the rescue medication is ready to use and a resuscitation set is available on the ward.

KEY REFERENCES

**Figure 2** Diagnostic approach to the patient with allergic rhinitis. (Reprinted from J Allergy Clin Immunol, 129/6, Rondon C, Campo P, Togias A, et al. Local allergic rhinitis: concept, pathophysiology, and management, 1460-1467, Copyright 2012, with permission from Elsevier.)
WHY DO WE DO FOOD CHALLENGES?
Diagnosis of food allergy can be obvious, for example in the case of a child reacting with urticaria 10 minutes after having eaten peanuts. In this case caused by an IgE-mediated reaction, skin prick tests and in-vitro diagnosis, by measuring specific IgE in the serum, are most often positive and clearly related to the symptoms. Food challenge might be done in patients with vague symptoms possibly related to food allergy, with a significant proportion of patients having positive IgE tests not related to the symptoms but to an atopic predisposition (false positive tests indicating only sensitization). In this context, a food challenge will be the gold standard for diagnosis (Figure 1).

HOW DO WE DO FOOD CHALLENGES?
Several variables have to be taken into account when organizing a food challenge. Most importantly, the food challenge must be designed in a safe way. The food challenge must be performed in a location, where emergency medication can be administered. When doing high risk challenges, it is important to have rapid access to intensive care facilities. In all cases, the food challenge must be supervised by nurses trained to recognize early signs of reactions. Also, the supervising physician must be trained in order to have sufficient experience in interpreting the various clinical signs possibly observed during a food challenge.

The food is provided to the patient in increasing doses. The starting dose, as well as the time interval between the doses, will be determined by the initial history of the patient and by the purpose of the challenge. A food challenge aiming to determine the threshold level of a reaction will start with a very low dose.

The food can be administered openly, but also in a double-blind placebo controlled manner. During this procedure, the challenge is separated into two parts, the food being hidden in a vehicle in order for blind both the patient and the examiner (Figure 2).

LIMITATIONS OF A FOOD CHALLENGE
A well designed food challenge will provide definite information about the presence or the absence of a food allergy. Nevertheless, one needs to be aware of some caveats. A food challenge is valid only for the type of preparation of the food. As an example, patients may react to raw egg, but not to cooked egg, and a patient with a negative challenge to cooked egg might afterwards react to a preparation with raw or not completely cooked egg. Similarly, a negative challenge to a specific fish does...
A well-designed food challenge is the definite diagnostic tool for food allergy. In addition, it is also a very useful tool in the follow-up of food allergic patients. A positive food challenge provides valuable information to the patient on how the reaction could take place and how to treat it, thus increasing the patient’s quality of life.

**KEY REFERENCES**


Oral provocation test (OPT) with aspirin (ASA) is the gold standard for the diagnosis of cross-reactive types of hypersensitivity to NSAIDs, which include NSAIDs-exacerbated respiratory disease (NERD), NSAIDs-exacerbated cutaneous diseases (NECD) and NSAIDs-induced angioedema/urticaria. In patients with single NSAIDs induced type of reactions, ASA is rarely a culprit drug, so OPT with the suspected NSAID may be performed. In patients with NERD, bronchial or nasal challenges with the soluble form of ASA (lysine-ASA) may be an alternative to OPT to confirm/exclude NSAIDs hypersensitivity.

**ORAL PROVOCATION TEST**

Oral challenge with ASA is recommended in patients with suspected respiratory or cutaneous form of reactions to NSAIDs. Appropriate safety measures should be followed and contraindications considered (Table 1). In patients with NERD, the two day protocol starts with placebo capsules administered on the first day, every 1.5-2 hours, with FEV1 measured every 30 minutes to establish baseline variability (Table 2). On the second day, the patient receives initially 10-30 mg of aspirin and the dose is doubled at 1.5 hours intervals, until a positive reaction occurs, defined by at least 20% fall in FEV1 and/or extra-bronchial symptoms (nasal or ocular congestion, rhinorrhea, erythema of the skin, gastrointestinal symptoms). The test is negative if none of the above appears and the final ingested dose of 312 mg of ASA is well tolerated. A similar protocol, with extended intervals between ASA doses is indicated for the diagnosis of the cutaneous type of NSAIDs hypersensitivity (Table 3).

**TESTING WITH OTHER NSAIDS**

OPT can be used for testing hypersensitivity to other culprit NSAIDs or to assess tolerance to an alternative NSAIDs. Even if no immediate indications for treatment with NSAIDs exist, the potential patient’s need for an analgesic, antipyretic or anti-inflammatory treatment should be anticipated. Tests with alternative NSAIDs usually start with the oral administration of half of the therapeutic dose followed by monitoring in the doctor’s office up to two hours.

**INHALATION PROVOCATION TEST**

In patients with NERD, inhalation or intranasal challenge with Lysine (L)-ASA (a soluble form of acetylsalicylic acid) can be used as an alternative to ASA-OPT (Figure 1). The bronchial provocation test start with the inhalation of a diluent, followed by increasing doses of L-ASA administered using
In vivo diagnosis of NSAID hypersensitivity

**TABLE 1**

Indications and contraindications to oral aspirin challenge in patients with NSAIDs hypersensitivity

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Ambiguous or not clear history of response to aspirin/NSAIDs</td>
</tr>
<tr>
<td>• Confirmation/exclusion of cross-sensitivity if aspirin is not a culprit NSAID</td>
</tr>
<tr>
<td>• Beginning of desensitization</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>• History of severe anaphylactic reactions induced by aspirin or other NSAIDs</td>
</tr>
<tr>
<td>• Not well controlled underlying disease (asthma/urticaria)</td>
</tr>
<tr>
<td>• Low baseline FEV1 (below 70% of the predicted value)</td>
</tr>
<tr>
<td>• Severe delayed type reactions</td>
</tr>
<tr>
<td>• Concomitant disorders potentially aggravated by the challenge</td>
</tr>
</tbody>
</table>

**TABLE 2**

Protocol for the oral aspirin challenge in a patient with suspected NSAIDs exacerbated respiratory diseases

<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>Placebo</td>
<td>10 mg</td>
</tr>
<tr>
<td>9:30</td>
<td>Placebo</td>
<td>27 mg</td>
</tr>
<tr>
<td>11:00</td>
<td>Placebo</td>
<td>44 mg</td>
</tr>
<tr>
<td>12:30</td>
<td>Placebo</td>
<td>117 mg</td>
</tr>
<tr>
<td>14:00</td>
<td>Placebo</td>
<td>312 mg</td>
</tr>
</tbody>
</table>

*a Optional in patients with a history of severe reaction to NSAIDs

**TABLE 3**

Protocol for oral aspirin challenge in a patient with suspected NSAIDs exacerbated cutaneous disease

<table>
<thead>
<tr>
<th>Time</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>Placebo</td>
<td>71 mg</td>
</tr>
<tr>
<td>10:00</td>
<td>Placebo</td>
<td>117 mg</td>
</tr>
<tr>
<td>12:00</td>
<td>Placebo</td>
<td>312 mg</td>
</tr>
<tr>
<td>14:00</td>
<td>Placebo</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

A dosimeter at 30 minutes intervals, allowing to complete the procedure in an outpatient setting in less than 5 hours. The test is considered positive, if at least 20% drop in FEV1 is recorded at 10, 20 or 30 minutes after inhalation. If the positive reaction is observed PD20 ASA is calculated from the dose-response curve. Inhalation challenge may induce extrabronchial symptoms and may also involve a late phase reaction. Both oral and inhaled tests have similar sensitivity and specificity but, as compared to the oral challenge, the inhalation test, is faster and safer to perform (the reaction is usually easily reversible with nebulized beta2 agonists).
### Advantages and Limitations of Oral, Bronchial, and Intranasal Route of Aspirin Challenge in Patients with NSAIDs-Exacerbated Respiratory Disease

<table>
<thead>
<tr>
<th>Route</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral challenge</td>
<td>Gold standard (reliable)</td>
<td>Risk of severe reaction</td>
</tr>
<tr>
<td></td>
<td>Useful in patients with extra bronchial symptoms</td>
<td>Time-consuming</td>
</tr>
<tr>
<td>Bronchial challenge</td>
<td>Short duration (&lt; 4 h)</td>
<td>Only in patients with bronchial/nasal symptoms</td>
</tr>
<tr>
<td></td>
<td>Reaction easily reversible</td>
<td>Requires special equipment (dosimeter)</td>
</tr>
<tr>
<td>Intranasal challenge</td>
<td>Safe in patients with severe/unstable asthma</td>
<td>Only in patients with bronchial/nasal symptoms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not possible in patients with nasal obstruction</td>
</tr>
</tbody>
</table>

**Figure 1** Advantages and limitations of oral, bronchial and intranasal route of aspirin challenge in patients with NSAIDs-exacerbated respiratory disease.

The nasal provocation test with L-ASA is particularly recommended in asthmatic patients with low pulmonary function not suitable for bronchial provocation. The challenge starts with instillation of saline followed by L-ASA solution increasing concentrations applied into each nostril every 30 minutes. All parameters (objective and subjective) are recorded every 10 minutes. Alternatively, ketorolac nasal spray solution can be used. For the assessment of the challenge, clinical symptoms are combined with the objective measures of nasal airflow patency (acoustic rhinometry, rhinomanometry or peak nasal inspiratory flow).

The reaction is considered positive, if clinical symptoms appear and/or a significant nasal obstruction is objectively documented (a 25% decrease in total nasal flow or 40% drop of the inspiratory nasal flow, as compared to baseline).

In experienced hands the sensitivity of intranasal aspirin provocation may rise above 80% and specificity approaches 95%, thus reaching the performance of the bronchial challenge.

The nasal challenge route is not recommended in patients with significant nasal obstruction, turbulent nasal flow or with unspecific nasal responsiveness.

**KEY REFERENCES**

BACKGROUND AND INDICATIONS
A drug hypersensitivity (DH) reaction is particularly difficult to prove, as the history may be unreliable and for many drugs and reactions no sensitization can be demonstrated by skin tests or in-vitro tests. A drug provocation test (DPT) is performed: a) to exclude DH in a non-suggestive history (e.g. unspecific symptoms arising after intake of a penicillin), b) if a cross-reactivity in proven hypersensitivity has to be excluded (e.g. to other pain medications in acetylsalicylic acid hypersensitivity), c) if a firm diagnosis in suggestive history of DH with negative, non-conclusive or non-available allergological tests shall be established (Table 1). It is considered to be the gold standard for DH diagnosis, required when other allergy tests do not allow relevant conclusions.

PROCEDURE
A drug provocation test (DPT) is the controlled administration of a drug for diagnostic purposes, performed under close medical surveillance with emergency medication available and always after an individual risk-benefit analysis. Requirements for a DPT include the possibility to hospitalize patients, experience with emergency treatment, consideration of pharmacological effects of tested drugs, exclusion of contraindications, informed consent of the patient and sufficient wash-out interval from anti-allergic medication. It is a high risk procedure as it induces symptoms similar to the original ones, albeit normally milder because of initially low and fractionated doses and immediate treatment (Figure 1, Table 2).

The oral provocation test is the preferred route for most DPTs, although subcutaneous (e.g. for local anaesthetics, heparins, insulins), local or even intravenous provocations (e.g. for heparin) are possible. The time interval between dose escalations and the

Figure 1. Mild generalized acute urticaria after oral drug provocation test with 500 mg acetylsalicylic acid (as in Aspirin®) in a patient with reported drug hypersensitivity to another potentially cross-reacting pain medication.
duration of provocation have to be
determined considering the origi-
nal reaction and suspected mech-
anism.

General guidelines for perform-
ing DPT have been published and
specific protocols are available for
some drugs, such as betalactam
drugs, nonsteroidal anti-inflam-
matory drugs or radiocontrast
media.

**INTERPRETATION**
In the majority of patients, DH can
be excluded by DPT. In some pa-
tients, the reappearance of reac-
tions confirms the diagnosis. False
positive and false negative results
to DPTs are possible. In medicine,
a DPT can never guarantee toler-
ance in 100% of patients, but helps
to decide, if lifelong avoidance is justified. Studies evaluating the valid-
ity of the DPT show that the ma-
jority of patients (>95%) toler-
ated the drug in real life, if there
were no reaction in the DPT and
conclude that this test is benefi-
cial for the patient.

**KEY REFERENCES**
2. Messaad D, Sahla H, Benahmed S, Godard P, Bousquet J, Demoly P. Drug provocation tests in pa-
tients with a history suggesting an immediate drug hypersensi-
3. Joint Task Force on Practice Para-
rameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunolo-
gy: Drug allergy: an updated prac-
tice parameter. *Ann Allergy Asthma

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**TABLE 1**

<table>
<thead>
<tr>
<th>Indications for a drug provocation test</th>
</tr>
</thead>
<tbody>
<tr>
<td>• To exclude hypersensitivity in non-suggestive history of drug hypersensitivity</td>
</tr>
<tr>
<td>• To exclude cross-reactivity of related drugs in proven hypersensitivity</td>
</tr>
<tr>
<td>• To establish a firm diagnosis in suggestive history of drug hypersensitivity with negative, non-conclusive or non-available allergological tests</td>
</tr>
<tr>
<td>• Validation of the history and other allergological tests (reference standard)</td>
</tr>
</tbody>
</table>


**TABLE 2**

<table>
<thead>
<tr>
<th>Clinical reactions to drug provocation tests in 241 patients with confirmed drug hypersensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manifestation</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Urticaria</td>
</tr>
<tr>
<td>Maculopapular exanthema</td>
</tr>
<tr>
<td>Bronchospasm</td>
</tr>
<tr>
<td>Laryngeal edema</td>
</tr>
<tr>
<td>Anaphylaxis without shock</td>
</tr>
<tr>
<td>Anaphylactic shock</td>
</tr>
</tbody>
</table>

In vivo allergy diagnosis - drug provocation test
The allergen challenge chamber

In contrast to several other provocation tests, allergen challenge chamber (ACC) tests are not focused on single organs, but on several targets of type I allergic reactions like eyes, upper and lower respiratory tract and even skin. In contrast to other allergy tests, ACCs are usually not applied for individual diagnosis, but for assessing allergic treatments, studies in occupational allergy and basic research.

At present, there are twelve ACCs active in Europe, USA, Canada and Japan (Figure 1). There is a broad conformity between the systems, although individual differences are to be mentioned. All ACCs are validated for pollen challenges like grass/ birch/ragweed or Japanese cedar pollen; some are also validated for house dust mite or even for cat (Figure 2).

The main common feature of all ACCs is a pre-defined and stable allergen concentration in the air, which has to be high enough to induce symptoms in sensitized subjects. In addition, the allergen concentration climatic conditions like temperature, humidity and CO2 concentration are kept stable over several hours. All parameters are constantly controlled to ensure high reproducibility of the ACC model. During a challenge session patients record their subjective symptoms several times an hour with good compliance. There is usually no application of rescue medication, which could possibly complicate the interpretation of the symptom score. Usually nasal, ocular and/or bronchial symptoms are scored using a 4-point scale (0 to 3).

A challenge session lasts at least two hours, because by then a plateau is normally reached in all of the patients’ symptoms, which will then not vary, if the allergen load is kept stable. This plateau-reaction is usually monitored for two to six hours (Figure 3).

In contrast to field or park studies, it is possible to additionally monitor and report objective assessments during the challenge sessions. These objective parameters offer the opportunity to check the properness and accuracy of the subjective scoring (Table 1). Furthermore, it is possible to take blood samples at several time points of the challenge session to follow the ongoing allergic reaction.

The ACC model is approved by the FDA and EMA, at least for phase II trials and further supporting phase III trials. EMA suggests ACC trials for conducting dose finding trials of immunotherapeutic products. Potential fields of application of ACC trials, however, can be more extensive (Table 2).
ACC can monitor specific immunotherapy and reduce the cost of drug development substantially, not only by proof of concept studies. Requiring significantly less time than field studies and smaller numbers of individuals per trial are the essential arguments for application of ACCs. Besides several advantages some disadvantages are to be mentioned (Table 3). The standardization of several ACCs worldwide and the “between-unit” reproducibility are still unmet targets.

**KEY REFERENCES**
1. Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM et al. The role of allergen challenge chambers in the evaluation of anti-allergic

**TABLE 1**

<table>
<thead>
<tr>
<th>Objective assessments performed in an ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinomanometry</td>
</tr>
<tr>
<td>Spirometry</td>
</tr>
<tr>
<td>Peak Expiratory Flow (PEF)</td>
</tr>
<tr>
<td>Nasal secretion weight</td>
</tr>
<tr>
<td>Nasal scraping</td>
</tr>
<tr>
<td>Nasal lavage</td>
</tr>
<tr>
<td>Nasal Peak Inspiratory Flow (NPIF)</td>
</tr>
<tr>
<td>Slit lamp investigation</td>
</tr>
<tr>
<td>Conjunctival imaging</td>
</tr>
<tr>
<td>Digital Endoscopy</td>
</tr>
<tr>
<td>Acoustic rhinometry</td>
</tr>
<tr>
<td>Blood samples (drug level, mediators...)</td>
</tr>
</tbody>
</table>

Figure 1 Distribution of Allergen Challenge Chambers in the World.

Figure 2 The Vienna Challenge Chamber (VCC)


Elevated serum levels of allergen-specific IgE demonstrate the atopic status of a patient and are indicative for an eventual clinically significant allergy.

Determination of allergen-specific IgE in serum allows rapid screening of the sensitization spectrum of a patient without risks for adverse reactions.

Multiplex specific IgE measurements to pure natural or recombinant allergens allow a component-resolved diagnostics, which can be useful to design a patient-tailored immunotherapeutic intervention.

For allergy diagnosis elevated allergen-specific serum IgE levels need to be interpreted in the light of clinical history. In some cases, in vivo provoked tests performed with the suspected allergen are needed to confirm the diagnosis.

The breakthrough allowing the development of highly specific and sensitive in vitro tests for allergic diseases was the discovery of the “reaginic antibody” termed immunoglobulin E (IgE) more than 40 years ago. The prototype for the in vitro detection of serum IgE (the radioallergosorbent test, RAST) first described in 1967 used a paper disc as a solid phase to covalently immobilize the allergen followed by the addition of patient’s serum. After different washing procedures to remove unbound serum proteins and antibodies, bounded IgE was detected with 125I-labelled polyclonal anti-human IgE (Figure 1). Modern assays for the detection of allergen-specific IgE have undergone impressive improvements including the calibration against the WHO Standard 72/502, allowing quantitative determinations, and the implementation of fully automated devices (Figure 2). To date the most commonly used system to determine allergen-specific IgE is the ImmunoCAP system (Thermo Fisher Scientific, Uppsala) considered as the “gold standard” for the in vitro diagnosis of allergic conditions. More recently, novel diagnostic tests based on allergen microarrays have been introduced both in research and clinical practice. Multiplex-based in vitro tools for allergy diagnosis allow a component resolved diagnostics of the atopy status of a patient in a cost effective way.

Figure 1  Typical immunoassay for the detection of allergen-specific IgE in three steps: 1) the allergen is adsorbed and immobilized to a solid phase, 2) patient’s serum is added followed by incubation during 30-60 min and several washing steps and 3) allergen-bound IgE is detected by an enzymatically labelled anti-human IgE monoclonal antibody ( ).
Despite the relevant technological improvement, several problems related to the in vitro diagnosis of allergy remain unsolved (Figure 3). Allergen-specific IgE in serum is a marker for sensitization and a prerequisite for the development of an IgE-mediated allergy, but is not sufficient for the induction of symptoms. In fact, more than 20% of the individuals with allergen-specific serum IgE are asymptomatic. We should be aware that allergen-specific serum IgE is biologically irrelevant as long as it doesn’t bind to the high affinity receptor for IgE on effector cells. Therefore, the presence or the absence of allergen-specific IgE in serum is not sufficient to confirm or exclude an allergy in all cases.

At the current state of the art, it is worthwhile to maintain a healthy scepticism about allergy diagnostic test results solely based on the determination of allergen-specific serum IgE levels and to let the clinical history, corroborated by adequate provocation tests, which drive the diagnosis before considering immunotherapeutic interventions.

**KEY REFERENCES**


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**Figure 2** Most commonly used techniques for the detection of allergen-specific IgE used in research and clinics. All techniques are based on the immunoassay principle described in Figure 1.

**Figure 3** Steps involved in the development of allergic diseases. A switch to the production of allergen-specific IgE detectable in serum leads to the sensitization of a patient, reflecting its atopic status. However, not all sensitized individuals suffer from allergic symptoms highlighting the limitation of in vitro allergy diagnoses. Allergen challenge tests are needed to confirm the clinical relevance of an allergy suspected from the clinical history and from the presence of allergen-specific IgE in serum. *(Reproduced with permission from Bousquet J, Anto JM, Bachert C, et al. Factors responsible for differences between asymptomatic subjects and patients presenting and IgE sensitization to allergens: a GA2LEN project. *Allergy* 2006;61:671-680, with permission from Willey Blackwell.)*

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The history of in vitro (laboratory) tests for allergy diagnosis started in the early seventies, soon after the discovery of IgE in 1967. The first generation assays, starting from the very first RAST, allowed researchers and allergists to improve the diagnosis of IgE-mediated diseases. The first generation IgE assays were mainly based on allergenic extracts – a mixture of allergenic and non-allergenic compounds derived from natural sources (e.g. a pollen extract).

A great step forward to a broader use of in vitro diagnosis in allergy was achieved in the early nineties, when the more reliable second generation assays appeared in laboratories worldwide and in parallel a new kind of test reagent started to be available. In fact, during the nineties an increasing number of allergenic molecules have been identified, characterized and, most important, progressively translated into the daily use in diagnosis (Figure 1A). Most of the available allergenic molecules have been obtained by using the recombinant DNA technology, allowing cloning and production of recombinant allergens in a reproducible large scale. Within the last decades, allergenic molecules have been increasingly used in research and clinical studies leading to a cumulative number of scientific publications (Figure 1B).

This knowledge is now displayed in several free accessible internet-based resources documenting the magnitude of the worldwide allergy health problem (Table 1). The most recent keystone improvement in this area has been the application of microtechnology to in vitro diagnosis of IgE-mediated diseases. In the last decade, a new tool has become available to allergists allowing them the parallel diagnosis on hundreds of allergenic molecules from a huge collection of allergenic sources in a single test run (Figure 2). This multiplex microarray test is now available worldwide and allows allergic patients to be tested with the same panel of allergens regardless of age, gender and kind of allergen and route of exposure.

Within the last decade, multiple studies from many countries around the world independently reported an added diagnostic value by using molecular components for in vitro allergy diagnosis in the singleplex test systems – the more traditional approach in allergy laboratory testing. This progress became mainly evident in some of the most severe allergic diseases such as anaphylaxis due to food intake or insect stings, which can result in fatal outcome. Through the advent of molecular technologies, some of the weaknesses and

**Key Messages**

- The advent of molecular allergen technologies has created a shift of paradigm in diagnosing IgE-mediated allergies
- Component-resolved analysis of the specific IgE response provides a more individualized and stratified diagnosis of allergic patients
- Component-resolved diagnosis (CRD) of the specific IgE response improves the sensitivity, specificity and clinical performance of the laboratory assay
- CRD has the potential to better select patients for immunotherapy, to predict the risk for severe allergic reactions and to monitor patients for immunotherapy outcome.
shortcomings of the classical diagnostic algorithm, which was solely based on allergenic extracts from nature, could be solved in terms of assay sensitivity, specificity and prediction of clinical severity and risk. Nowadays, several prominent examples exist, where the measurement of sIgE to molecular allergen components is a recommend-ed state-of-the-art procedure within the algorithm for clinical decision-making (Table 2).

From a global perspective – despite the immense progress that has been achieved in the field of molecular allergy in vitro testing within the last two decades – there is a clinical and research need for a better reflection of allergenic molecules based on regional differences in the world. Not all allergens are global, some of them are regional, but still can cause severe allergic disease. This new era of allergy diagnosis, which calls for a global definition of allergenic source and molecules, has already been entered. The analysis of such wealth of data is now possible by using advanced information and communication technologies, which will ultimately result in better care for allergic patients.
TABLE 1
Comprehensive allergen databases providing free access.

<table>
<thead>
<tr>
<th>Database</th>
<th>URL</th>
<th>Target/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO/IUIS Allergen Nomenclature Sub-Committee</td>
<td><a href="http://www.allergen.org/">http://www.allergen.org/</a></td>
<td>Responsible for maintaining and developing a unique, unambiguous and systematic nomenclature for allergenic proteins</td>
</tr>
<tr>
<td>ALLERGOME</td>
<td><a href="http://www.allergome.org/">http://www.allergome.org/</a></td>
<td>A web site designed to supply information on allergenic molecules (allergens) for clinicians and researchers</td>
</tr>
<tr>
<td>AllergenOnline</td>
<td><a href="http://www.allergenonline.org/">http://www.allergenonline.org/</a></td>
<td>Provides access to a peer reviewed allergen list and sequence searchable database</td>
</tr>
<tr>
<td>AllFam</td>
<td><a href="http://www.meduniwien.ac.at/allergens/allfam/">http://www.meduniwien.ac.at/allergens/allfam/</a></td>
<td>The AllFam database is a resource for classifying allergens into protein families (based on the Allergome website)</td>
</tr>
</tbody>
</table>

Abbreviations and explanations:

TABLE 2
Molecular components or allergens with a demonstrated utility for clinical decision making in various allergic conditions

<table>
<thead>
<tr>
<th>Allergic Disease</th>
<th>Molecular Component / Allergen</th>
<th>Diagnostic Problem Solved</th>
<th>Countries of Major Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insect venom anaphylaxis</td>
<td>Api m 1, Ves v 1, Ves v 5, Pol d 5</td>
<td>Better differentiation of honey bee and vespid venom double-sensitized patients</td>
<td>GER, SUI, AUT, SLO, USA, ITA, POL, ESP</td>
</tr>
<tr>
<td>Insect venom anaphylaxis</td>
<td>Ves v 5</td>
<td>Increased sIgE assay sensitivity through spiking of vespid venom with rVes v 5</td>
<td>GER, SUI, AUT</td>
</tr>
<tr>
<td>F/WDEIA</td>
<td>Tri a 19</td>
<td>Marker allergen for food-/wheat-dependent exercise-induced anaphylaxis (F/WDEIA)</td>
<td>JAP, FIN, DAN, GER, SUI</td>
</tr>
<tr>
<td>Peanut allergy</td>
<td>Ara h 2, Ara h 6</td>
<td>Predictive marker allergens for severe reactions to ingested peanut</td>
<td>USA, GBR, GER, AUS</td>
</tr>
<tr>
<td>Allergy to red meat</td>
<td>α-Gal</td>
<td>Marker allergen for delayed anaphylactic reactions to red meat and offal</td>
<td>USA, SWE, GER, AUT</td>
</tr>
<tr>
<td>Pollen Allergy; Insect venom anaphylaxis</td>
<td>CCD</td>
<td>Marker for broad IgE-based cross-reactivity without clinical relevance</td>
<td>ITA, NED, GER, AUT</td>
</tr>
<tr>
<td>Insect venom anaphylaxis</td>
<td>Api m 10, Api m 3</td>
<td>Prediction of patients at risk for therapeutic failures in honey bee venom immunotherapy</td>
<td>GER, SUI</td>
</tr>
</tbody>
</table>

Abbreviations and explanations:
Tri a 19, wheat allergen α-5 gliadin; α-Gal, mammalian carbohydrate determinant (galactose-α-1,3-galactose); CCD, carbohydrate cross-reactive determinants (β-1,2-xylose and α-1,3-fucose); Api m 1, 3, 10, honey bee venom allergens Phospholipase A2, Acid Phosphatase, Icarapin; Ves v 1, 5, vespid venom allergens Phospholipase A1, Antigen 5; Pol d 5, Polistes venom allergen Antigen 5; Ara h 2, 6, major peanut allergens storage protein allergens.

KEY REFERENCES

In vitro allergy diagnosis – molecules and component-resolved diagnosis
Cellular allergy testing is essentially induction of the allergic response in a test tube. As any in vitro test, it has the advantage of quantifying the allergic response without any risk to the patient.

The most common cellular test applied is basophil activation by allergen (BAT) (Figure 1). This is used when the patient history and specific IgE or skin tests are discordant, when there is no reliable specific IgE or skin test, or if the patient history indicates that skin tests may elicit a systemic response. Determination of basophil sensitivity with serial dilutions of allergen can be used to measure the point of inflection or half maximal allergen concentration as measure of allergic response. This is a more reproducible measure of allergic disease than provocation testing. Basophil sensitivity can be used to identify food allergens, the primary sensitizer amongst cross-reacting allergens or allergen preparations and to monitor progress of allergen immunotherapy and anti-IgE therapy (Figure 2).

Basophil activation is measured as either histamine release or as upregulation of granule proteins to the cell surface in response to allergen exposure. The most common antigen to be measured on the surface of blood basophils, which are identified as CD193 or CD203c positive cells in whole blood is the tetraspannin, CD63. CD63 resides in the same vesicles as histamine in blood basophils and in tissue mast cells.

For optimal performance, introduction of basophil activation including setting up the flow cytometer should be done in the context of the existing network of units offering this service. There is a strong movement toward standardisation of measurement of basophil activation by flow cytometry, embodied amongst others by the EAACI-EuroBAT meetings and an EAACI Task Force on this topic. Contribution to and complying with standardisation will enhance the quality of this recent diagnostic tool for allergy diagnosis.

Type IV reactions to drugs can be severe and it is important to identify the eliciting allergen in these patients. This is done with the lymphocyte transformation test, but increasingly also by Elispot and with flow cytometric techniques. These analyses remain in the hands of specialised units that deal with these severely afflicted patients.
Figure 1  Basophil activation in clinical diagnosis of allergy to celery. Patient (left) with negative (green) and positive (blue) controls, and allergen response in red is reactive. A tolerant control person is shown on the right (unpublished).

Figure 2  Anti-IgE treatment monitored with basophil sensitivity to allergen. Before treatment the patient was extremely sensitive (red) and after two months of treatment both sensitivity and reactivity had decreased (green). Rubak and Hoffmann, unpublished.

KEY REFERENCES
Biomarkers are used to diagnose diseases, estimate disease severity, or investigate underlying inflammation types, thus predicting the efficacy of therapeutics. For example, measuring a specific IgE or urinary leukotriene E4 is important for diagnosis of atopic status or aspirin-sensitive asthma, respectively. Discovering novel biomarkers, as well as finding appropriate combinations with existing biomarkers, is an important goal in allergy research aiming to improve diagnosis and treatment.

“Companion diagnostics”, which can predict therapeutic efficacy, is now being widely recognized due the development of molecular targeted drugs against allergic diseases, particularly asthma. These drugs, mostly biologics, are expensive and are effective only for particular types of patients. It is now thought that asthma is not a single disease, but a “syndrome.” Thus, it is important to classify asthma into several endotypes, based on their pathogenesis, and evaluate for which endotype, using the appropriate biomarkers each molecular targeted drug is effective using the appropriate biomarkers.

Although inhaled corticosteroids (ICS) provide beneficial effects for most asthma patients, 5-10% of patients are resistant or hypo-responsive to ICS. Any biomarker indicating the cause of the resistance to ICS would be useful. Moreover, biomarkers that can predict asthma relapse upon steroid reduction would be also valuable. Eosinophilic airway inflammation is at least one factor associated with steroid responsiveness. Fractional exhaled nitric oxide (FeNO) is considered a surrogate biomarker for eosinophilic airway inflammation and is regarded as a biomarker for steroid responsiveness (Figure 1). Although still controversial, the accumulated evidence has suggested that FeNO is useful for assessing control with ICS, predicting relapse upon ICS reduction, and estimating lung function.

Periostin, a downstream molecule of IL-4 and/or IL-13, has emerged as a surrogate biomarker of type 2 inflammation and tissue remodelling in bronchial asthma. It has been shown that serum periostin can predict the efficacy of anti-IL-13 antibody (lebrikizumab) and anti-IgE antibody (omalizumab), thus proving a prototype biomarker for the companion diagnostic of allergic diseases (Figure 2). Moreover, high serum periostin is linked to hypo-responsiveness for ICS, particularly in late-onset eosinophilic asthma (Figure 3). In addition to periostin, sputum eosinophils are useful for estimating the efficacy of anti-IL-5 antibody (mepolizumab).
Genetic biomarkers, such as the filaggrin gene for skin barrier dysfunction or the glucocorticoid-induced transcript 1 gene for responsiveness to steroid therapy, represent another avenue to be explored in allergy research.

**Figure 1** A treatment guideline for adult asthma patients based on existing symptoms and FeNO levels (Modified from Ref. 4)

**Table 1**

<table>
<thead>
<tr>
<th>FeNO</th>
<th>&lt;25 ppb</th>
<th>25-50 ppb</th>
<th>50&lt; ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms (+)</td>
<td>Consider alternative diagnosis</td>
<td>Uncontrolled</td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Symptoms (-)</td>
<td>Well controlled</td>
<td>Consider taper or withdrawal</td>
<td>Well controlled</td>
</tr>
</tbody>
</table>

**Figure 2** Serum periostin as a biomarker to select Th2 antagonists for asthma patients. Upper panel: Responsiveness in asthma patients to Th2 antagonists depending on serum periostin is depicted. Patients showing high serum periostin are responsive to anti-IL-13 antibody (lebrikizumab) and anti-IgE antibody (omalizumab), whereas those showing low serum periostin are unresponsive to these agents. (Summarized from Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, Harris JM. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011 365; 12, 1088-1098, Hanania NA, Wenzel S, Rosen K, Hsieh HJ, Mosesova S, Choy D F. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. Am J Respir Crit Care Med 2013 187; 804-811) Lower panel: An algorithm of treatment of asthma patients is shown. The first choice for asthma patients is ICS. If the patients are resistant to ICS, measurement of serum periostin is recommended. If serum periostin is high, Th2 antagonists are recommended; if low, other agents are recommended.

**KEY REFERENCES**


Figure 3 Serum periostin as a biomarker to predict responsiveness to ICS in asthma patients. Upper left panel: Serum periostin levels in rapid and non-rapid FEV1 decliners are depicted (8). Upper right panel: Clustering of asthma patients based on peripheral neutrophils and eosinophils is depicted. Lower left panel: Characteristics of each subtype are shown. Lower right panel: Correlation between serum periostin and responsiveness to ICS in cluster 3 is depicted. (Reprinted from J Allergy Clin Immunol, 133/5, Nagasaki T, Matsumoto H, Kanemitsu Y et al. Integrating longitudinal information on pulmonary function and inflammation using asthma phenotypes, 1474-1477, Copyright 2014, with permission from Elsevier.)

Biomarkers for allergy diagnosis and treatment