

Diagnostic Reagents for Type I Allergy – What Criteria Should Be Applied to Validation?

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Introduction

Type-I-allergies belong to the most prevalent chronic diseases worldwide which include anaphylaxis as well as the initiation of severe chronic diseases. The World Health Organization considers allergic diseases to be a major public health problem. Allergic rhinitis often is the first step in the development of allergic asthma, the sequel often being a mixed bronchial asthma. Anaphylaxis to insect stings (honey bee and *Vespula* species) may occur in 5%, to allergenic food in 3% and to natural rubber latex in 1% of the population. Deaths have mainly been reported as a sequel to insect stings and the ingestion of allergenic food. The only causal anti-allergic therapy is the allergen-specific immunotherapy. The accuracy of diagnosis is essential for either the avoidance of the culprit allergen or the correct decision for an efficacious treatment.

In order to detect the causative allergen(s), which should be used for treatment, an exact differentiation has to be made between mere sensitization and the clinically relevant IgE-reactivity (allergy) to the allergen in question. Sensitization to a certain allergen is characterized by an allergen-specific IgE reactivity without an effector cell response and, therefore, not accompanied by clinical symptoms, whereas allergy is defined as clinically relevant allergen-specific IgE reactivity due to the capacity of IgE-antibodies to cross-link FcεRI receptors on effector cells.

Trigger identification, evidence of an IgE-antibody-mediated sensitivity and association of this sensitivity with symptoms, which can be identified with obvious clinical history and allergen testing are the basis of diagnostic measures of allergic diseases. *In vitro* IgE-antibody-detection only proves sensitization, and skin-prick-test (SPT), being considered in this presentation as representative example for procedures like intracutaneous application via injection or solutions for provocation, basically shows sensitization although an adequate diagnostic procedure consists of the synopsis of

the two diagnostic procedures and the patients' allergy history to evaluate the clinical relevance (Figure 1). The latter can be proven by provocation tests directed on the organ-target (nasal, bronchial or oral challenges) with the offending allergen.

Skin-Prick-Test

Skin prick test (SPT) is a cutaneous test for *in vivo* diagnosis of type I-hypersensitivity against allergens, as are the allergen extract application via intracutaneous injection or provocation tests. This presentation focuses on SPT as an apt representative example. For SPT, a minimal quantity of allergen is introduced into the epidermis of the volar forearm by means of a sterile lancet without penetrating the dermis. Histamine and sodium chloride (0.9%) are used as positive and negative controls, respectively. The readout is performed after 15 to 20 min. In case the patient is sensitized to the allergen in question a weal and flare develops as result of the reaction between the allergen contained in the SPT-solution and the patients' immune system. Immediate hypersensitivity reactions are initiated when allergen molecules cross-link the Fab-components of adjacent IgE-molecules on the IgE-receptors on the surface of effector cells, i.e. mast cells, followed by the release of preformed mediators like histamine, proteases, and proteoglycans from secretory granules as well as newly produced eicosanoids and cytokines. Two reactions can be observed after SPT with an allergen extract: an immediate and a late-phase reaction of which the first is the most important reaction with regard to the application of allergen extracts as diagnostic tools in allergy. It is dependent on the degranulation of mast cells immediately after the allergen challenge, thereby inducing the typical weal and flare reaction of the skin.

When performing skin tests (Table 1), it is important to use test extracts with an appropriate allergen concentration in order not to induce false-positive or false-negative reactions. Tests should not be performed within the first week after anaphylactic reactions as there may be a refractory period. In cases where commercial test substances are not available (some foods, some occupational substances), tests must also be performed in non-exposed non-allergic controls.

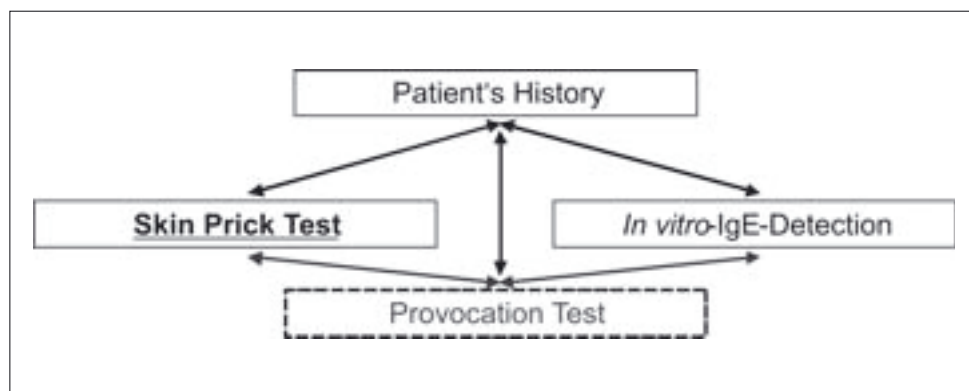


Fig. 1. Synopsis of diagnostic procedures and patients' history to evaluate clinical relevance

Table 1. *SPT - An IgE-based primary method to specifically identify type I-hypersensitivity*

Indications	Conformational test in cases where history of allergic symptoms already gives a reasonable suspicion for a particular allergen to be the cause of the allergic disease Screening test in cases where history provides some evidence for the existence of an allergic disease with unknown agents. Confirmation of the diagnosis and determination of the intensity of the sensitization prior to the initiation of specific immunotherapy with hymenoptera venoms. Test to determine sensitization as part of epidemiological studies Determination of the biological activity of allergen extracts Determination of efficacy and kinetics of topically and systemically applied drugs
Contraindications	Skin disease in test area Poor general condition Uncontrolled bronchial asthma Tests associated with the risk of a severe (systemic) reaction: beta-blocker treatment* Pregnancy*
Value	Good reproducibility Minimally invasive Mechanism of action is limited to the application site No subsequent liberation, absorption or distribution of the allergen extract into the blood stream Good concordance with clinical symptoms Not dependent on age inexpensive
Limitations	Detection of sensitization and no proof of allergy Cross-reactivity Concentration optimum generally unknown Accuracy of skin testing is limited in drug allergies. Allergen extracts currently used for SPT are prepared from natural allergen-sources and may give highly variable results [1].
Risk factors	History of previous anaphylactic reactions Uncontrolled asthma Allergen contact right before SPT-performance [2] SPT with highly concentrated allergens [3] SPT with raw material, i.e. fresh foods [4, 5] SPT with hymenoptera venoms SPT with antibiotics Beta-blocker treatment [6] Subpopulations at risk: small children, pregnant women, patients with a high degree of reactivity [7]
Developments	Validation against standardized challenge tests: predictive value [8] Standard SPT throughout Europe (GA2LEN) [9] SPT-reliability assessment in the European Community Respiratory Health Survey (ECRHS-I) [10] Correlation between SPT using commercial extract of cow's milk protein and fresh milk and food challenges [11] Correlation between SPT reactivity to purified allergens and clinical severity [12] Purified allergens versus complete extract in the diagnosis of inhalant allergy [13] Natural allergen sources versus recombinant technology [14], recombinant diagnostic as well as therapy allergens: a useful treatment monitoring tool? Standardization of allergens, i.e. CREATE [15] and BSP 090

* An exception can be made in case the SPT-result is essential for a treatment decision and a systemic anaphylactic reaction is not to be expected when considering all data.

SPT is inexpensive, easy to perform and provides a result within a few minutes. The number of allergen extracts available for diagnostic purpose include materials derived from many allergen sources such as pollens, venoms of stinging insects, latex, moulds, mites, animal dander, antibiotics, anesthetics, and foods.

Readout

SPT- read-out should be performed according to the recommendations of the scientific societies. The positivity criterion is the area of the weal. The SPT is positive with a mean weal diameter of $\geq 3\text{mm}$ [16]. Because the reaction to histamine shows individual differences it is advised not to use the comparison with the histamine reaction as parameter for SPT positivity. The diagnostic value of the SPT is warranted only in cases where the negative control induces no reaction at all and the histamine control produces a clearly positive weal and erythema reaction.

Although it is rare, false positive reactions may occur, for example in cases of urticarial dermatographism, cutaneous mastocytosis or after application of extracts that contain additional irritants. False negative reactions are possible as well in case extracts with initially very small amounts of allergen are used or if the reactivity is suppressed by drugs (see below). Young children and elderly may have reduced skin reactivity.

Prior and concomitant therapy

Some drugs are known to interfere with the weal size, and it is essential to consider their half-life before testing. H(1)-receptor antagonists are known to suppress reactions in SPTs. However, the effect of H(2)-receptor antagonists, which are widely used in everyday practice, remains unclear. Because the sensitivity and specificity of skin testing requires withholding medication that could change the skin reactivity, it seems important to take into account the possible influence of H(2)-receptor antagonists on allergy diagnosis and therapy [17]. Further drugs and their half-life have to be taken into account: ketotifen, glucocorticosteroids, imipramine, amitriptylin, desipramine, promethazine and intravenous immunoglobulins.

For both, allergen preparations for specific immunotherapy and diagnosis of sensitization by skin tests, the extracts must be well characterized and standardized. The according regulatory guidance has recently been revised [18,19]. A new guideline on allergens for specific immunotherapy has come into force in 2009 [20]. The necessity for standardization of allergen extracts regarding their allergenic activity and potency has been recognized long ago and continuous efforts are ongoing to improve their quality.

SPT: Why is it so important to have good test allergens, and what problems are there?

There are not enough diagnostic allergen(s) (extracts) available for allergologists, particularly regarding food and occupational allergens. Authorized extracts are prepared from natural sources and may give highly variable results. Not all relevant

Table 2. *List of the most relevant regulatory guidance documents*

- The German Medicinal Products Act
- The Guideline on allergen products: Production and Quality issues (EMA/CHMP/BWP/304831/2007)
- Monograph on Allergen Products, European Pharmacopoeia 01/ 2010:1063 (EDQM)
- Guideline on the Clinical Development of Products for Specific Immunotherapy for the treatment of Allergic Diseases CHMP/EWP/330611/2008.
- Points to Consider on the Evaluation of Diagnostic Agents CPMP/EWP/1119/98
- Notice to applicants A GUIDELINE ON SUMMARY OF PRODUCT CHARACTERISTICS (Draft 2009)
- GUIDELINE ON THE READABILITY OF THE LABELLING AND PACKAGE LEAFLET OF MEDICINAL PRODUCTS FOR HUMAN USE Revision 1, 12 January 2009
- GUIDELINE ON RISK MANAGEMENT SYSTEMS FOR MEDICINAL PRODUCTS FOR HUMAN USE EMA/CHMP/96268/2005
- VERORDNUNG (EG) Nr. 1901/2006 DES EUROPÄISCHEN PARLAMENTES UND DES RATES vom 12. Dezember 2006 über Kinderarzneimittel und zur Änderung der Verordnung (EWG) Nr. 1768/92, der Richtlinien 2001/20/EG und 2001/83/EG sowie der Verordnung (EG) Nr. 726/2004
- DIRECTIVE 2001/83/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 6 NOVEMBER 2001 ON THE COMMUNITY CODE RELATING TO MEDICINAL PRODUCTS FOR HUMAN USE

allergenic components are present in solutions provided for SPT (i.e. lipid transfer proteins). Therefore, clinicians still depend on the application of raw materials. This procedure is associated with the risk of adverse events due to the fact that the allergen concentration is unknown. In the past, the design of studies for marketing authorization application has not been ideal. As far as the standardization of allergens for diagnosis and treatment is concerned there is still a lot of work to do and the respective studies are still ongoing.

Regulatory control of diagnostic allergen products (Table 2)

The basis of the regulatory control of diagnostic (and therapeutic) allergen products in Europe is the Directive 2001/83/EC of the European Parliament and of the Council of November 6, 2001 on the Community Code Relating to Medicinal Products for Human Use (Official Journal L – 311, 28/11/2004, p. 67–128). This directive defines as immunological medicinal product “any medicinal product consisting of vaccines, toxins, serums or allergen products: ... (b) ‘allergen product’ shall mean any medicinal product which is intended to identify or induce a specific acquired alteration in the immunological response to an allergising agent.”

Additional relevant guidance for allergen products is provided in the German Medicinal Products Act, the “Guideline on Allergen Products: Production and Quality Issues” (EMA/CHMP/BWP/304831/2007 [18]), the “Monograph on Allergen Products”, European Pharmacopoeia 01/2010:1063 (EDQM) [19], and the

“Guideline on the Clinical Development of Products for Specific Immunotherapy for the treatment of Allergic Diseases” CHMP/EWP/330611/2008 [20]. The “Note for Guidance on Allergen Products” (CPMP/BWP/243/96) has been substituted by the new BWP-guideline EMEA/CHMP/BWP/304831/2007 [18]. The “Leitallergenprinzip” is no longer valid but has been substituted by the “*Concept of Homologous Groups*” [21]. The Paediatric regulation 1901/2006 mandates that all applications for marketing authorization submitted after July 26, 2008, must be supported by an approved Paediatric Investigation Plan (PIP).

As this publication primarily focuses on the clinical aspects of the regulation of SPT-solutions rather than on pre-clinical or quality issues, the respective problems are mentioned only briefly. Issues often criticized are the quality of the allergen extracts, the fact that applicants use their own in-house-references and their own protocols for standardization. Furthermore, the choice of in-house-reference sometimes is not ideal in the respective context or its validity is not documented. Data are often inaccurate with regard to the precise allergenic activity of the test allergen preparations, the stability of the extracts, allergen content, and/or the concentration of major allergen(s). However, some questions have still remained open, i.e. the consideration of minor allergens as being of importance, and what the apt potency test might be. The revised BWP-Guideline EMEA/CHMP/BWP/304831/2007 will give a more detailed guidance regarding quality issues.

The mode of action of SPT-preparations is limited to the area where they are applied. Subsequent liberation, absorption or distribution of the allergen extract into the blood stream is most probably not to be expected. Unmodified allergen extracts represent substances which naturally occur in the environment and to which the total population is exposed. There are no relevant animal models, the allergen concentration applied when using SPT-solutions is very small, and there are no suitable detection methods in order to perform pharmacology studies. Therefore, pharmacokinetic and pharmacodynamic studies with allergen extracts have not been considered to be necessary. There is a lot of clinical experience in humans, and unnecessary animal studies should be avoided.

A detailed discussion of the literature should be provided including the list of drugs that are known to interfere with the SPT result (see above). Conventional toxicology studies are considered not to be necessary for unmodified extracts of substances, which naturally occur in the environment and to which the total population is exposed. In general, a statement should accompany the application as to whether the investigations were performed according to good laboratory practice (GLP). However, the regulatory requirements are different with regard to modified and recombinant allergens.

Regulation of Skin Prick Test Solutions

In 2001, Prof. D. Haustein, former Director of the Division of Allergology, Paul-Ehrlich-Institut, has made a still valid proposal for the requirements of studies for allergens which considers the grade of difficulty in recruiting the respective patients

(Tables 3a, 3b). These represent internal criteria of the Paul-Ehrlich-Institut for test allergens and are applicable for national procedures. They are, however, not documented in European Guidelines.

So far, the limitations of clinical studies submitted to the competent authority mainly were due to the lack of well-characterized patients as documented by accurately designed and completed case report forms (CRFs), the lack of dose finding studies, and of correct definitions as far as sensitization and allergy as well as SPT-positivity is concerned. As a result, often merely sensitized but not truly allergic patients have been enrolled. The choice of a comparator has not always been ideal, and mostly there has been no control group.

A. Study design

In general, a double-blind placebo-controlled prospective design is preferred. Open studies have so far been accepted. However, it is essential to include a control group.

B. Choice of population

The following aspects should be taken into account for the choice of population:

- A concise definition of allergy symptoms with regard to the general allergy history and particularly the allergen under investigation should be provided. The CRF should consist of detailed information on allergy symptoms and potentially culprit allergens, onset, duration and treatment of symptoms as well as concomitant treatment.
- A description of the study population should be provided.
 - ▶ I. patients allergic to the allergen(s) in question
 - Patients allergic to the allergen in question should have a clear and consistent history of the respective allergic symptoms. It is preferred to have an additional detection of IgE-antibodies specific for the allergen in question.
 - ▶ II. control group consisting of non-allergic, SPT- and allergen-specific IgE-antibody negative individuals (*preferred*) or
 - individuals allergic to allergens other than the study substance, non-allergic and non-sensitized to the allergen under investigation

Table 3a. *Categories of Diagnostic Allergens in Germany (valid since 2001 (Prof. Dr. D. Haustein))*

Clinical Criteria for Proof of Efficacy	Marketing Authorization			
	A	B	C	D
Prospective Clinical Trials	+	-	-	-
Statistical Evaluation of Skin Test Charts (Retrospective Evaluation)	-	+	either	-
2 or 3 single Case Reports (Documentation of the Number of Patients tested)*	-	-	or	-

* Condition: Confirmation of Patient's Allergy History via SPT with the Extract in Question

Table 3b. Examples of allergens and the according categories

Allergen	A	B	C	D
Birch <i>Betula verrucosa</i>	+			
Oak <i>Quercus</i> spp.		+		
Hazelnut Tree <i>Corylus avellana</i>		+		
Olive Tree <i>Olea europea</i>		+		
Cypress <i>Cupressus</i> spp.		+		
Willow Tree <i>Salix</i> spp.			+	
Juniper <i>Juniperus communis</i>				+
Rye pollen <i>Secale cereale</i>	+			
Ray Grass <i>Lolium perenne</i>	+			
Timothy Grass <i>Phleum pratense</i>	+			
Honey Grass <i>Holcus lanatus</i>				+
Apple, Peanut, Hazelnut		+		
Celery, Soy Bean, Shrimp			+	
Grapes				+

- Ideally, patients should be recruited from a geographical area, where the allergen under investigation occurs with high prevalence.
- Diagnostic tests prior to enrolment should not only include basic safety parameters but also allergen-specific IgEs to the most prevalent inhalant allergens as well as the respective SPT.
- Provocation tests are considered the gold standard because they are ideal functional tests to confirm allergy towards the allergen in question. However, as not every study center has the necessary experience or equipment, they are not obligatory.
- In case of test allergens for which clinical relevance cannot be proven, i.e. alder, a positive history of symptoms in the relevant season and the detection of allergen-specific IgE-antibodies are obligatory.

C. Definition of inclusion and exclusion criteria (Table 4).

D. Choice of a comparator

As a comparator, an SPT-solution of the allergen in question already authorized or raw material in case of foods should be used. When raw source material is used, healthy controls should be included in any case.

E. Calculation of sensitivity and specificity

A concise and justified definition of true positive and true negative SPT-results should be provided by the applicant. Ideally, an SPT-result is considered true positive if it is positive in patients allergic to the substance under investigation and true negative if it is negative in healthy individuals. The same is considered justifiable if SPT-results are correlated with the respective challenge test.

F. Dose finding studies

The study design can be open and non-randomized. The number of patients should be chosen in accordance with bio-statistical recommendations. Three to four steps of a three- or ten-fold dilution should be applied subsequently and in duplicates. Inclusion and exclusion criteria are as provided in Table 4. As test read-out the criteria documented above are to be applied.

Post-marketing experience is appreciated as source of supportive data.

Conclusion

Standardized and high-quality diagnostic allergens are a valuable diagnostic tool enabling allergologists to accurately diagnose their patients because treatment decision and control depend on the correct diagnosis. SPT as diagnostic tool is under complex development: SPT as monitoring parameter for allergen-specific immunotherapy, SPT as substitute for dangerous challenge tests, investigation of SPT with

Inclusion Criteria	Exclusion Criteria
• Patients capable to sign informed consent • Allergy to the allergen(s) in question: <i>ideal</i> • Sensitization to the allergen(s) in question • Definition of age groups • Both sexes • Positive SPT ≥ 3mm with a standardized extract prepared from the allergen source in question and / or • a positive test for allergen-specific IgE-antibodies • Positivity of the positive control (histamine dihydrochloride) • Negativity of the negative control i.e. sodium chloride 0.9%	• Immunotherapy in the past 2 years with an allergen preparation known to interfere with the allergens to be tested. • Use of drugs that may interfere with SPT-results (see contraindications) • Poor general condition • Uncontrolled asthma • Pregnancy • Dermographism • Atopic dermatitis (locally at the test site) • Urticaria • Patients with contraindications for treatment with adrenalin (hypertension, thyroideal disease, heart disease, beta-blocker treatment) • Participation in another clinical trial within the last 2 months

Table 4. SPT-Study design: Inclusion and exclusion criteria

recombinant allergens as potential surrogate marker, and SPT as integral part of component-resolved diagnosis, to name only a few research activities.

Aims that can be achieved at present are the improvement of the quality of allergens used for SPT-preparations as well as the quality of clinical studies. Regulatory documents have been created and up-dated in order to provide applicants with the respective guidance. Diagnostic allergen preparations are essential: Clinicians, applicants (companies) as well as the competent authorities should see to it that they gain on quality.

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Discussion

de Blay: Thank you for your talk, it was very interesting. What will be the consequence of these four categories for skin prick tests? I mean, when you are “D”, you want to have a “C” and then “B”. What do you think will be the future, will the “D” be allowed at all?

Jappe: No, the categories were developed in order to give some guidance and also reflect the availability of patients. What we have been discussing about was to change the categories now, because so much development has taken place and there are more patients available with, let us say, a celery allergy. So, we believe that prospective clinical trials can be done, which was not the case before.

Vieths: This is not an official regulation, it is an internal procedure, an internal document and an agreement we have with manufacturers. The idea is that for those allergens where you have very few patients, but still the extract is needed or requested by the clinicians, you can register the product just with quality data and without any clinical data. That is the idea. But if we are in the situation, as Uta mentioned, where there are 100 challenged patients published in the literature we would probably put the product in a higher category even if the company claims that it is impossible to find enough patients.

André: Congratulation for your presentation but I have two concerns about your exclusion criteria. One is pregnant women. You know the status in Europe was that it was a well-tolerated immunotherapy, even injections could continue during pregnancy. So it is quite clear that nobody will start immunotherapy with a pregnant woman. But I think that skin prick tests could be very useful simply as an eradication or prevention measure, both for pneumo- and food allergens. And the other concern I have, thinking with all my heart, is that you are thus excluding patients who are

on beta blockers. It is quite clear that beta blockers will make it more difficult to respond to adrenaline in case of emergency. This is something that you can read in any kind of book, but it is not absolutely proven. In fact, it is exactly the same with drugs that are able to dramatically increase the effect of adrenaline by increasing the blood pressure after injection in a very dangerous manner. So I wonder if we should be so strict about those criteria.

Jappe: Largely, I agree with you. What I wanted to point out was that the moment we perform trials with very strong allergens in dose-finding studies, we probably should not include pregnant patients and those under beta blocker therapy. As far as treatment is concerned there is agreement about the fact that when there is, e.g., a hymenoptera venom therapy that has been performed before the woman became pregnant, the therapy should of course be continued due to vital reasons.

Kleinjans: Can you tell me where your personal opinion conflicts with the statements of the PEI? Because at the beginning, you said that you are giving your own opinions and not the PEI opinions. I was now thinking about the difference between your own and the PEI opinion?

Jappe: I think Prof. Vieths should comment on that, but it is mainly that the PEI opinion really is official and has gone through several steps of development, ending, let us say, in a guideline or so.

Mäkinen-Kiljunen: I just wanted to ask you what was the concentration of histamine, because you have 10 mg/ml and 1 mg/ml. And think 10 mg/ml is okay, but you had also this 1 mg/ml.

Jappe: Yes, there is a controversy about that in the literature. So sometimes it said 0.1 % and sometimes it is ...

Mäkinen-Kiljunen: I think it is a European Academy recommendation that 10 mg/ml should be used, it is more or less a rule.

Jappe: Yes, you are right. In this case, I took the information from the S2 guideline which in this case obviously does not conform to the European one. Sorry about that.