# ART AND SCIENCE OF PERCUTANEOUS SKIN TESTING

**Course 1605**

AAAAI Annual meeting, Orlando, 3 – 5 pm, March 2nd, 2012

**Sten Dreborg**
Professor, MD, PhD, FAAAAI
Department of "Women's and Children's Health"
University of Uppsala
SE-751 85 UPPSALA
Sweden

Tel.: +46 302 511 93; Mobile/Cellular: +46 7 33 42 33 41;
E-Mail: sten.dreborg@kbh.uu.se; or sten.dreborg@telia.com

**Please observe!**

## Page 2-3 should be printed in advance

by all participants in Workshop 1605A-4: ***Art and Science of Percutaneous Skin Testing***, Friday March 2nd, 2012. AAAAI Annual Meeting, **Orlando**.

# Proficiency testing: Skin prick test / Percutaneous skin test

## Background

The precision of the skin prick/puncture test is not optimal.

It is essential to minimize the variation making it possible to relay in the test results.

In 500 patients tested with 3 allergen concentrations in quadruplicate one in twenty tests were negative in some clinics, and many more were found to be "out-layers" with responses much smaller than the average. That means that when only single tests with each allergen are performed, then many test results are read much smaller than they should be. In case correct response is a small wheal, then many tests performed by an assistant with high cv., i.e. a big variation in size of wheals with the same extract in the same patient, may be negative, i.e. false negative.

The precision of the skin prick test is low, i.e. the coefficient of variation, the c.v. is high. In university allergology outpatient departments the precision varies, calculated on the area, between 40 and 140 % and when calculated on the basis of the diameter of the wheal between 20 and 70 % (Area = $\pi r^2$, i.e. the cv. of the area is twice that of the diameter). Common in vitro tests have a c.v. less than 10 %.

# Methods

To check the precision, there are two possibilities:

To perform

**Duplicate tests with all allergens and the positive control, histamine**

The same needle can be used for both tests with the same allergen, but the method is more time-consuming. The pricks are somewhat painful. This is inconvenient in small children.

**The duplicates should have the same size, i.e. ± 1 mm in diameter.**

**Twenty tests with histamine on the volar aspect of the forearms once a month**

Ten tests with histamine should be performed on the volar aspect of both forearms. Tests should be done at monthly or bimonthly intervals in 3-4 individuals. Whatever brand or concentration of histamine used in your clinic/office can be used.

The wheal diameters or areas should be registered and read by an independent person and the c.v. calculated.

**The diameter should not vary more than ± 1 mm in diameter.**

## The hands on session

You are welcome to try different methods, material will be supplied by the congress.

# Proficiency testing with histamine

## Right Arm

| | | | | | |
|---|---|---|---|---|---|
| 1 | | D =… | 6 | | D =… |
| 2 | | D =… | 7 | | D =… |
| **3** | | D =... | 8 | | D =... |
| **4** | | D =... | 9 | | D =... |
| **5** | | D =... | 10 | | D =... |

Start of testing:

15 min at:

**Mean:**

Range:

c.v

## Left arm

| | | | | | |
|---|---|---|---|---|---|
| 1 | | D =… | 6 | | D =… |
| 2 | | D =… | 7 | | D =… |
| 3 | | D =... | 8 | | D =... |
| 4 | | D =... | 9 | | D =... |
| 5 | | D =... | 10 | | D =... |

Start of testing:

15 min at:

**Mean:**

Range:

c.v.

**Fill in to the right of each registered wheal**

$$\frac{d_{longest} + d_{orthogonal\ mid}}{2} = D$$

**Give the mean wheal diameter**, the size of the **largest and the smallest wheal** diameters (range, should not be more than ± 1 mm)

Alternatively the c.v. can be calculated for diameters per patient and per testing personnel.

This test record should be **printed by all participants prior to the workshop**.

# Skin prick testing

## Introduction

The clinical history is the basis for the diagnosis of allergy.

Sensitization can be shown by *in vitro* IgE tests or by *in vivo* tests, mostly skin tests. Skin prick testing is regarded the standard method for diagnosis of allergen sensitization.

**Sensitization vs. clinical allergy**
Although sensitization always implies a risk of clinical symptoms, the difference between IgE-sensitization and clinically important IgE-mediated allergy is practically important.

**Mechanisms**
All allergies are not IgE-mediated. Despite allergic diseases have similar or identical clinical phenotypes; they may be caused by both atopic i.e. IgE-mediated mechanisms, and non-atopic, mainly cellular immunologic mechanisms [(1)].

**Diagnosis of clinical allergy**
In food allergic patients the diagnosis should be established by double blind placebo controlled food challenges (DBPCFC)[(2;3)] In young children, an open challenge can be performed, provided positive tests are verified by DBPCFC[(2)] .

In clinical trials the mucosal sensitivity to aeroallergens is sometimes proven by airway allergen challenges. However, in practice the diagnosis of allergy towards aeroallergens is mostly not confirmed by upper or lower airway challenge tests. Then, the diagnosis is relying on the technical cut off of the test used for the diagnosis of sensitization, but is not proving clinically important allergy, that is left to the judgment of clinicians.

Personally I never start immunotherapy or elimination procedures without a positive provocation test, for upper airway disease, a conjunctival provocation test by stepwise increasing doses. It is simple, rapid and convincing [(3ab)].

#### Definition of allergy

According to the definitions proposed by a working group within European Academy of Allergy and Clinical Immunology (EAACI)[(4)] and adopted by the World Allergy Organization, WAO[(5)] allergy is defined as an immunologically mediated hypersensitivity to foreign substances, allergens. The best defined mechanism is the interaction between allergen and allergen specific IgE, as-IgE, attached to receptors ($Fc_{\varepsilon}RI$) on mast cells (MC) and basophiles (BPh)[(6)]. However, immunologically mediated hypersensitivity can also be mediated by other mechanisms, mainly cell mediated mechanisms (Figure 1)[(4)].

#### IgE-mediated allergic diseases

Immunologically mediated hypersensitivity is named allergy (Figure 1)[(4;5)].

Some allergies are mediated by IgE antibodies, whereas others are mediated by other mechanisms, i.e. non-IgE-mediated allergy.

#### Atopy

Some of the IgE-mediated allergies are induced by very low quantities of inhaled or ingested proteins, allergens. These allergies are called atopic. Thus atopy is defined as the presence of IgE antibodies towards allergens, typically pollen, mite, animal dander and mold proteins.

**Allergic diseases**

Eczema (atopic dermatitis), asthma, allergic rhino-conjunctivitis etc. have been named "atopic diseases", but should be called "Allergic diseases". Allergic diseases can be either atopic, i.e. IgE-mediated, or induced by another mechanism, mostly by cells, i.e. non-atopic (Figure 1)[4;5]. Furthermore, IgE-antibodies are involved in allergic but not atopic diseases such as helminthic infections.

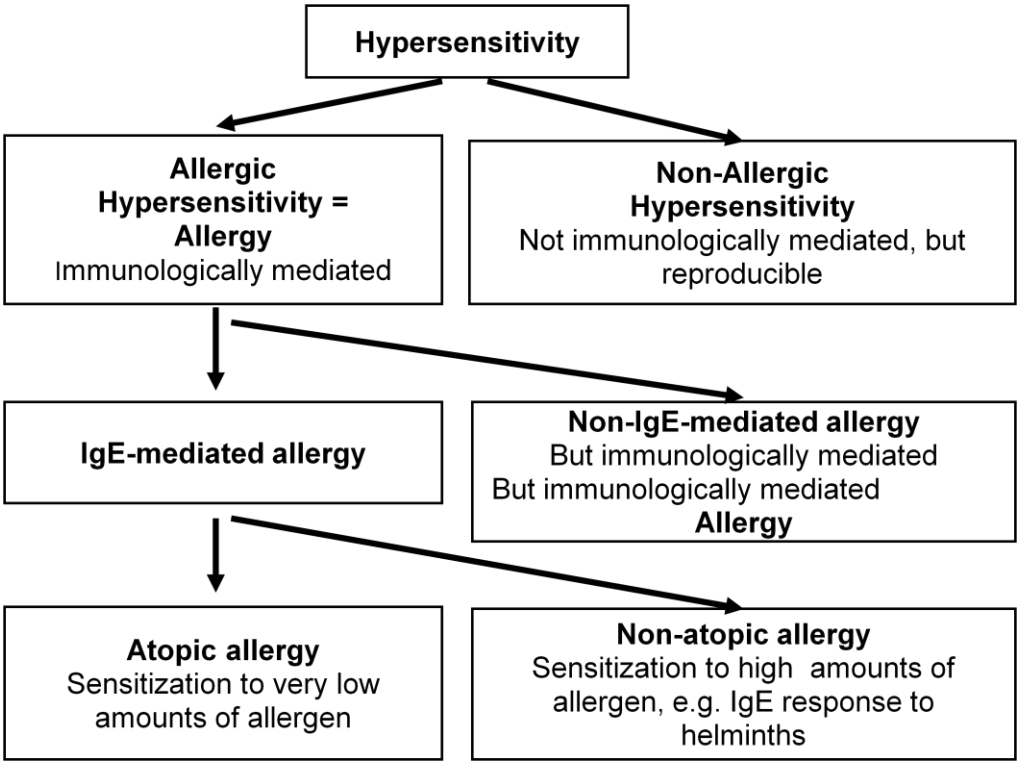



**Figure 1**[4;5]

Atopic sensitization means an as-IgE response to low concentrations of allergen.

# Skin prick testing

**Methods for skin testing**

Skin prick/puncture testing represents an easy way to demonstrate sensitization in a clinical setting. It induces an immediate wheal and flare reaction when using high concentrations of allergen, sometimes followed by a late reaction i.e. induration and erythema, appearing after some hours and lasting for hours to days.

Skin testing measures the combination of the presence of as-IgE, MC releasability, and tissue sensitivity to mediators in the skin.

The reaction is obvious to the patient and is therefore valuable to visualize the reaction to e.g. cat, dog or cockroach that makes it easier to the patient/parents to accept the allergy. It appears within a few minutes that makes it attractive in clinical practice. However, the method has many problems, which must be handled correctly, especially in scientific trials, but also considered in practice[7].

**Allergens for in vivo testing**

Allergen extracts used for *in vivo* diagnosis are often marketed as standardized, but most companies use ”Fantasy Units”[8] i.e. extracts are standardized against an in-house reference, IHR[8-10]. Each company has its own IHR and often company-specific source material. In some cases, the content of the most important allergens, major allergens, is also given.

However, the methods for determination of major allergen vary between manufacturers[10]. Thus, the content of major allergen cannot be compared between manufacturers[10]. The CREATE project[11;12] will hopefully find monoclonal antibodies and natural or recombinant allergens which in combination can be used by all laboratories to determine major allergens.

Furthermore, allergen extracts/allergens used for *in vivo* testing must be kept under conditions assuring stability. Enzymatic degradation of allergens is most pronounced among molds[13], but proteolytic enzymes are also present in pollen extracts[14].

A potent Cladosporium extract looses much of its allergenic activity within 3 months in glycerol and within one week in albumin diluent[13] So far, natural or recombinant allergens are not marketed for *in vivo* diagnosis of sensitization/clinical allergy.

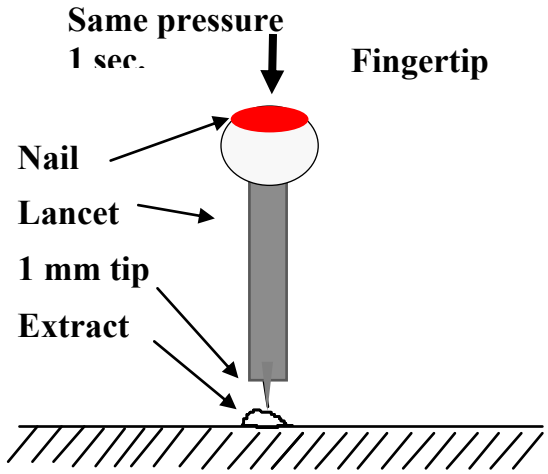
**Test devices**

Charles Blackley[15]did the first skin test on his own lower leg by scratching the skin and applying a dough of pollen. The reaction was immense with local swelling and itching that lasted for days. Thus, it included both an immediate and a late phase response that were not separated.

The skin prick test (SPT)/puncture test was introduced by Jack Pepys[16] in 1972. An epidermic needle was introduced trough a drop of allergen extract applied on the surface of the skin into the superficial layer of the epidermis, the tip was elevated, lifting the skin, allowing a minute amount of the extract to get into contact with skin MC.

Thirty years ago a **lancet with a 1 mm sharp tip and shoulders** preventing the lancet to penetrate more than 1 mm was introduced by Østerballe and Weeke, ”The allergy Pricker”[17]. The tip should e 1 mm with shoulders. Other features are of less importance [17].The tip of the lancet should be introduced vertically into a drop of extract on the skin, and pressed into the skin, using the volar aspect of the fingertip. The pressure should be the same every time (Figure 2), since, as for all similar devices, the response depends on the pressure applied.

The same principle applies to several other devices marketed today.




The technique using the "Allergy Pricker" has been shown to be better reproducible than that using the epidermic needle [18]. However, the precision varies between testers/nurses/assistants within wide ranges.

Therefore, **each testing person should use the technique he/she is currently using**, provided the c.v. is at an acceptable level, i.e. within ± 1 mm in diameter, or when calculated to be less than 20 % based on the wheal diameter, 40 % based on the wheal area.

**Figure 2.**

**Multi-test devices** have been introduced to simplify skin testing[19-21]. Plastic arms are attached to a central plate, each arm ending in a plate with a number of 2-3 mm long, sharp, plastic tips. Drops of allergen solutions are applied to the tips and the device is pressed against the skin. The advantage of multi-test devices is said to be convenience to the testing personnel. The major drawback is the high c.v. in the hands of some investigators [19]. I principle the length of the tip(s) etc of devices are the same as for single tip devices.

#### Skin response

The skin response to SPT is a wheal and flare reaction. The wheal size reaches a maximum after 15 to 20 minutes. In some patients the immediate reaction is followed by an allergen dose and as-IgE dependent late reaction[24;25], beginning within an hour and lasting for hours to days. The late response can be large and painful, but not dangerous. The late response is not used for diagnosis but for scientific studies.

An erythema should be present.

> The increase of the erythema diameter is more rapid than that of the wheal area and therefore recommended by FDA for scientific purposes (Peter Creticos workshop).

#### Recording of wheal diameter or area

It is recommended to record the test result 15 minutes after the test [26, 37]. The contour of the wheal should be drawn with a fine filter tip pen or ball point pen on the flare close to the wheal margin. The diameters can be measured directly on the skin (Figure 3), but it is recommended to transfer the drawing to a registration sheet by means of a translucent tape. Then the diameters or area can be calculated. The latter method preserves the wheal drawing for future comparisons.

The longest and the midpoint orthogonal diameters of the wheal (and/or flare) are added and the mean wheal diameter calculated by dividing by two. Most wheals less than 5-6 mm in diameter (about 30 $mm^2$) are more or less circular. The mean of two diameters, i.e. the mean of two plus two radians, and the area, *i.e.* squared radian times π, are associated. Only the error of measuring the diameters and the area may influence the end result.

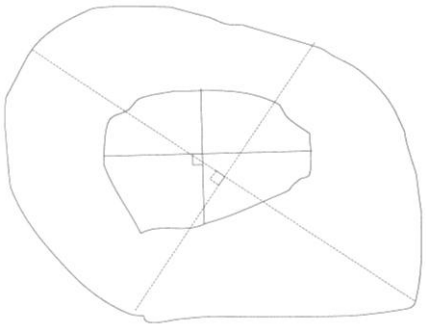


**Figure 3**

Inner drawing: The contour of the wheal.

Outer drawing: The contour of the Flare.

The area is measured or the longest and the midpoint orthogonal diameters of the wheal are added and the mean wheal diameter calculated by dividing by two.

To calculate wheal areas, planimetric[27;28] and scanning techniques[29] have been used.

Recently laser doppler imaging[30] digital photographic registration[31] of wheal areas have been tried. So far none of these techniques have been used in clinical practice. However, the basic problems remain, the high c.v. of the technique used by the technician, the potency and the composition of the extract used for testing[7]

**The dose response of the immediate wheal reaction**

The dose response of the wheal reaction is flat and best fitted to a log/log model: Log D or A = a + b log C (D is the diameter, A the area of the wheal, a, the intercept with the Y-axis, b, the slope and C the concentration of allergen extract). b, using the D is 0.19 for allergen. b, using A is 0.38 (95 % c.l. 0,37 – 0.40) for allergen and 0.34 (95 % c.l. 0,33 – 0.36) for histamine (Figure 4)[27;32].

When the concentration of the allergen extract is increased stepwise by ten times, then the wheal diameter increases from 2 to 3 mm, from 3 to 4.5 or from 4.5 to 6.8 mm in diameter, or the wheal area from 3 – 7 $mm^2$, from 7 – 11 $mm^2$, due to the flat dose response relationship of the wheal response to allergen.

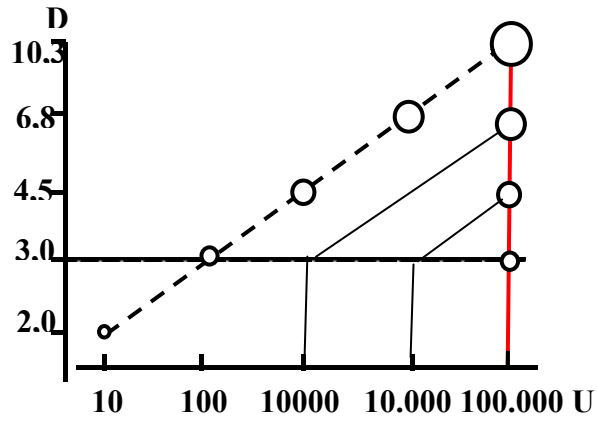



**Figure 4**

Vertical line: Wheal sizes in patients with 10-fold difference in sensitivity tested with the same extract.

Broken line: Wheal sizes in the same patient tested with ten-fold dilutions of one extract.

Thin lines: The calculated concentration eliciting a wheal with 3 mm D.

**The dose response of the immediate flare reaction**

The dose response of the flare reaction is steeper than that of the wheal reaction[33], but not used for evaluation of the reaction to SPT, but is used by Turkeltaub et al. for evaluation of the response to intradermal testing[34](Workshop by Peter Creticos).

**Concentration of allergen causing a defined wheal size**

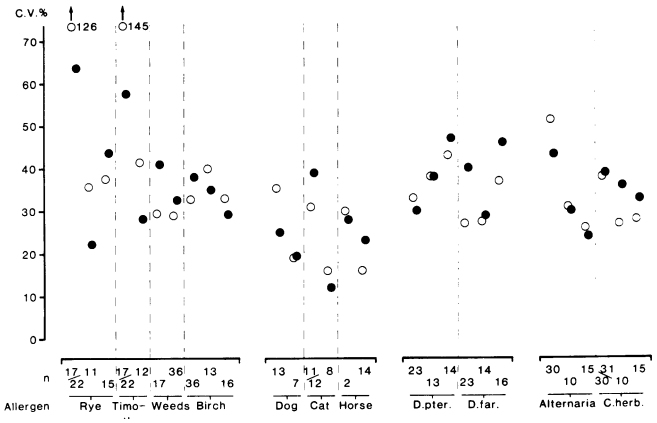
Bronchial sensitivity to methacholine, histamine, or allergen is normally given as the $PC_{20}$ or $PD_{20}$, i.e. the concentration or cumulative amount of methacholine, histamine, or allergen that causes 20 % reduction of $FEV_1$. The result of nasal and conjunctival provocation tests is also given using the threshold concentration causing symptoms.

Skin prick tests are most often performed with one concentration of allergen, supplied by the manufacturer (red line in figure 4). Probably therefore, the skin sensitivity is nowadays given as the diameter or the wheal area obtained with that single concentration. It is possible to calculate differences between groups based on the wheal size, but the data cannot be compared to threshold conc. of other organs[35].

However the concentration eliciting a certain wheal size, e.g. the same as that induced by the positive control, histamine, can easily be calculated by parallel line bio-assay(Figure 4)[35]. However, the same calculation can be made using the common slope of the dose response relationship (b), i.e. b = 0.39[27], according to the model: log Area = a + b log Concentration, or b = 0.19 using the diameter[27;32].

**Precision of the SPT and IDT.**

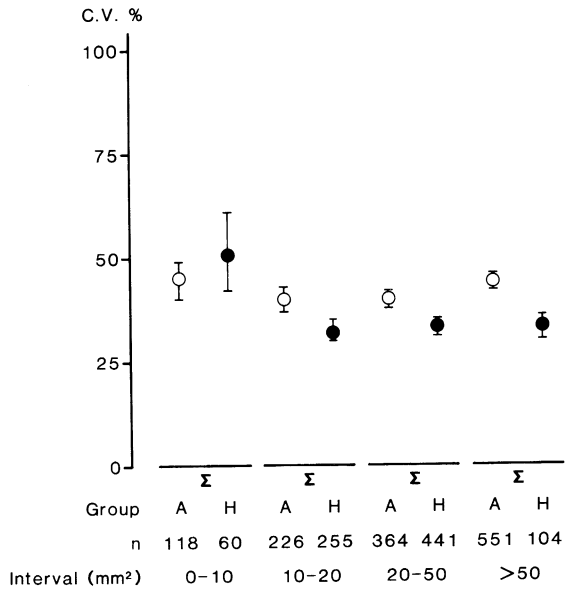
In relation to *in vitro* tests, the precision of SPT is poor[27]. The c.v. determined on the mean wheal diameter is, in most reports, ≥ 20 % and calculated on the area of the wheal ≥ 40 %. The precision of the IDT based on the wheal area is much better ≤ 10 %[36].




However, the c.v. of the SPT method varies considerably between centers (Figure 5)[27] and is higher at low response levels (Figure 6)[27].

## Figure 5

The c.v. of allergen wheal areas in a number of allergy clinics participating in biological standardization of common inhalant allergens. Some clinics with high c.v. tested grasses (from[27]). n = number of tested adults.




## Figure 6

The c.v. of allergen (A) and histamine HCl 10 mg/ml (H) wheal areas of different sizes largely corresponding to 10-fold increases in extract potency. Subjects were tested with three concentrations of the same allergen and histamine HCl 10 mg/ml all in quadruplicate[27].

A = allergen; H = histamine 10 mg/ml; n = number of patients.

**To reduce the high c.v. of the SPT, it is essential to use a proficiency program** in daily practice as well as in diagnostic and epidemiological studies using SPT.

**SPT proficiency testing**

One method is the use of SPT with all extracts in duplicate. Then, the sizes of duplicate tests, which should be identical, can be compared and these comparisons give immediate feedback to the assistant whether his/her technique is acceptable or not.

**As a rule of thumb**, the size of the two wheals obtained with the same extract should not differ more than ± 1 mm in diameter. The mean histamine wheal size should be the same from time to time to make it possible for you to compare results from time to time.

In studies it is this also important to make it possible to compare results with allergens between similar groups and within the same group over time. This method is inconvenient to young children.

Another possible method to document the precision of the method, in the hands of the assistant, is to perform a number of tests with the histamine reference used in your office in a reference group of patients or employees. These tests should be repeated at monthly intervals, the size of the histamine wheals should not differ between test occasions, and there should not be more than a ± 1 mm difference between the largest and smallest wheal diameter in the same patient.

This means that the c.v. should be less than 20 % based on the wheal diameters or 40 % when calculated on wheal areas, respectively. Furthermore the sine wheals should be documented and stored for future comparisons.

Due to the variable results between testing personnel, the skin prick test is not suitable for comparison of the sensitivity of patient over time or between regions.

In scientific trials for diagnosis of sensitization or allergy, it is mandatory to use some kind of proficiency evaluation[7;26], to minimize errors caused by varying test techniques within and between assistants involved in the trial.

**The cut off limit of the skin prick test**

In both North American and European position papers[26;37], the cut off has been set to 3 mm in diameter, i.e. 7 $mm^2$. There are several reasons for this:

- The dose response of the wheal diameter/area is flat,
- The high c.v. of the SPT method. The c.v. is high independent on whether it is calculated on the basis of diameters, areas, or if it is calculated on the concentration eliciting a given skin response, *e.g.* a wheal of the same size as that of the histamine wheal.[26-28]
- The measurement of small wheal areas/diameters is difficult and therefore the c.v. of small wheal areas/diameters is higher than that of larger wheal areas/diameters.
- The difference between 2 and 3 mm wheal diameter means a ten-fold difference in skin sensitivity.
- Instead of using non-precise measures of skin sensitivity, actually under the methodological detection limit, the potency of the test extract can be increased 10 or 100 times to detect slight sensitization - if wanted and needed. Several mg of allergen extract as measured by dry weight or protein, or about 1 mg of major allergen/ml have not shown irritating properties, provided endotoxins and the like are removed.

The cutoff of a laboratory diagnostic method is defined by the + 3 s.d. of the background response. In the case of SPT this has not been documented scientifically. It should be done by testing a high number of patients with many (at least 10) tests with the diluent, saline or dry device and then calculate the + 3 s.d. of the background, i.e. the trauma as such.

#### Sensitivity of the SPT

According to Turkeltaub et al[33] the ID test is about 1,000 times more sensitive than the SPT method. Niemeijer et al[38] found 30 Nordic BU/ml (about 30 ng of major allergen/ml[23] used for IDT to induce a wheal response of the same size as that of 3,000 BU/ml (about 3,000 ng of major allergen/ml[23] used in SPT, i.e. a 100-fold difference in sensitivity of the two methods. However, much higher concentrations must be used, *i.e.* at least 100,000 ng of major allergen, to diagnose most clinically sensitive patients[39] (with reduced specificity), due to the wide variation in sensitivity between patients sensitive to the same allergen source material. Actually, among patients sensitive to the same inhalant allergen, there is a difference in skin sensitivity of 1,000,000 times, i.e. an extract with 1 ng of major allergen/ml induces a skin response in the most sensitive patient but the least sensitive patient may need an extract with 1 mg of major allergen/ml to get the same reaction[40].

**For safety reasons, these concentrations must be considered when using new substances for testing** [41;42].

Intradermal skin testing inherits a higher risk of general reactions and fatalities [43;44] due to the much higher volume injected into the skin when testing intradermally than by SPT. If an initial SPT is negative or questionable, then the sensitivity of the SPT can be increased by increasing the concentration of allergen. When testing with new, non-documented substances or extracts, the initial concentration tested must be low, i.e. at the ng level of major allergen/ml. Then the concentration can be increased tenfold when using the intradermal route and 100 to 1000-fold when testing by SPT. If an SPT has a diameter < 3 mm, then a 1000-fold higher concentration will induce a wheal with at the most 10 mm diameter. If you prefer to go from SPT to IDT then the same concentration as used for negative or questionable skin response with SPT can be used for IDT without any risk of general reactions. However, when considering switching from SPT to IDT, it is important to use at least duplicate tests in SPT, to reduce the risk that the SPT is accidentally negative due to bad technique. Furthermore, when testing new substances the non-specific irritating effect must be checked, preferably by testing non-allergic, non-exposed individuals, with the same concentrations of allergen, obtaining negative results.

#### Annual variation in skin and organ sensitivity

The response to SPT varies in relation to pollen seasons[45], with stronger reactions after the season, slowly declining until before the next season indicating the boosting effect of allergen exposure. This is also true for the conjunctival provocation test[46]. Therefore, the season must be considered when reporting on sensitization or changes in skin response to allergen. However, both the SPT[47] and the CPT[48] give repeatable results within one month, i.e. correctly performed using reliable methods.

#### Affinity

It has been claimed that the affinity of IgE antibodies interfere with the results of not only *in vitro*[49] but also *in vivo* tests[6;50] contributing to explain the overlap between positive and negative tests in clinically sensitive patients.

## Variation of sensitization over time, between regions and samples as measured by SPT

A prerequisite for reliable results when comparing skin prick test results over time in the same population or between samples/populations is that the following criteria are fulfilled:

1. The same standardized extract, preferably freeze-dried, with the same potency and composition should be used. The content of major allergen(s) should be given as well as the method for determination of major allergen content.
2. The assistants/nurses should be trained in advance to use the same pressure on the lancet until the response to histamine HCl dihydrochloride 10 mg/ml (54.3 mmol/L) in a sample of individuals is constant. If histamine dihydrochloride 10 mg/ml is used, then the wheal diameter should be about 5-6 mm, ± 1 mm, in mean diameter.
3. The same assistant/nurse performs the test and her technique is documented as below.

4. In the study, at least duplicate tests should be performed, allocated mirrored, one set on each arm, or in small children, at least duplicate tests with histamine. The duplicates may not differ more than ± 1 mm in diameter.
5. If not duplicate tests can be performed, e.g. in small children, then at least 20 tests with histamine should be performed in a sample of individuals at regular intervals to compare the size of the wheal response to histamine from time to time and the c.v. of the histamine tests, obtained by the testing the same group of individuals/personnel.
6. Another person, not knowing the allocation of tests should read the test results.
7. The c.v. should always be reported
8. If different assistants/nurses are used in the same or different centers within the same trial, then they should perform tests in 10 individuals at the same time. At least ten tests with histamine dihydrochloride 10 mg/ml, i.e. 54.3 mmol/L, on each individual. The comparison between the participating curses should be repeated at intervals or at least at the end of the study using the same sample of patients/epmployees before and after the trial

There are very few studies that fulfill these criteria.

Criterion 1 is only fulfilled in a few major studies e.g. the worldwide ISAAC study [51] performed in several hundred centers using the same method. The same allergens were used in all centers. However, the methodology was not compared between all centers. In most trials conclusions have been drawn, despite different persons have tested different extracts have been used and no proficiency studies have been done.

# References

(1) Muraro A, Dreborg S, Halken S, Host A, Niggemann B, Aalberse R et al. Dietary prevention of allergic diseases in infants and small children. Part I: immunologic background and criteria for hypoallergenicity. Pediatr Allergy Immunol 2004; 15(2):103-11.

(2) Muraro A, Dreborg S, Halken S, Host A, Niggemann B, Aalberse R et al. Dietary prevention of allergic diseases in infants and small children. Part II. Evaluation of methods in allergy prevention studies and sensitization markers. Definitions and diagnostic criteria of allergic diseases. Pediatr Allergy Immunol 2004; 15(3):196-205.

(3) Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004; 59(7):690-7.

(3a) Moller C, Bjorksten B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. Allergy 1984; 39(1):37-41.

(3b) Dreborg S. Conjunctival Provocation Test (CPT). Allergy 1985; 40(Suppl 4):66.

(4) Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001; 56(9):813-24.

(5) Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004; 113(5):832-6.

(6) Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. Mol Nutr Food Res 2006; 50(7):620-4.

(7) Dreborg S. Skin testing in Allergen Standardization and Research. In: Dolen WK, editor. Skin testing. 2001: 329-54.

(8) Dreborg S. Standardization of allergenic preparations by *in vitro* and *in vivo* methods. In Dreborg S. & Frew A. (Eds.) Position Paper. Allergen standardisation and skin tests. Allergy 1993; 47(Suppl. 14):48-82.

(9) Einarsson R, Dreborg S. Manufacturers' criteria for in-house references. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1987;(80):131-8.

(10) Larsen JN, Dreborg S. Standardization of allergen extracts. Methods Mol Med 2008; 138:133-45.

(11) Chapman MD, Ferreira F, Villalba M, Cromwell O, Bryan D, Becker WM et al. The European Union CREATE Project: A model for international standardization of allergy diagnostics and vaccines. J Allergy Clin Immunol 2008.

(12) van RR, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O et al. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. Allergy 2008; 63(3):310-26

(13) Aukrust L. Rapid enzymatic degadation of allergens in Cladosproium extrats recostituted with glycerol or albumin diluent. Personal Communication, 1985.

(14) Bousquet J, Guerin B, Hewitt B, Michel FB. Analysis of commercial pollen extracts by enzyme determination. I. Comparison of rast-inhibition assay and enzyme titration for orchard grass, rye grass and short ragweed pollen extracts. Ann Allergy 1980; 45(5):310-5.

(15) Blackely C. Experimental researches on the causes and nature of catarrhus aestivus (Hay-fever and Hay-asthma). London: Ballière, Tindall & Cox, 18(16)

(16) Pepys J. Skin tests for immediate, type I, allergic reactions. Proc R Soc Med 1972; 65(3):271-2.

(17) Osterballe O, Weeke B. A new lancet for skin prick testing. Allergy 1979; 34(4):209-12.

(18) Basomba A, Sastre A, Pelaez A, Romar A, Campos A, Garcia-Villalmanzo A. Standardization of the prick test. A comparative study of three methods. Allergy 1985; 40(6):395-9.

(19) Demoly P, Bousquet J, Manderscheid JC, Dreborg S, Dhivert H, Michel FB. Precision of skin prick and puncture tests with nine methods. J Allergy Clin Immunol 1991; 88(5):758-62.

(20) Nelson HS, Rosloniec DM, McCall LI, Ikle D. Comparative performance of five commercial prick skin test devices. J Allergy Clin Immunol 1993; 92(5):750-6.

(21) Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. J Allergy Clin Immunol 1998; 101(2 Pt 1):153-6.

(22) Belin L, Einarsson R, Halvorsen R, Holgersson M, Lund B, Lofkvist T et al. Phazet™ - A New Type of Skin Prick Test Calibration and Stability. Allergy 1985; 40, Supplement 4:80-3.

(23) Dreborg S, Einarsson R. The major allergen content of allergenic preparations reflect their biological activity. Allergy 1992; 47(4 Pt 2):418-23.

(24) Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. J Allergy Clin Immunol 1985; 76(5):729-34.

(25) Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J Clin Invest 1976; 58(2):408-20.

(26) Dreborg S.& Frew A. Position Paper. Allergen standardisation and skin tests. Allergy 1993; 47(Suppl. 14):48-82.

(27) Dreborg S, Basomba A, Belin L, Durham S, Einarsson R, Eriksson NE et al. Biological equilibration of allergen preparations: methodological aspects and reproducibility. Clin Allergy 1987; 17(6):537-50.

(28) Dreborg S. The skin Prick Test. Methodological studies and clinical applications. Linköping University, Dissertation 239, 1987.

(29) Pijnenborg H, Nilsson L, Dreborg S. Estimation of skin prick test reactions with a scanning program. Allergy 1996; 51(11):782-8.

(30) Brink M, Malmkvist LA, Dreborg S. Laser doppler imaging (LDI) of the histamine skin prick test wheal and flare reaction. Personal Communication.

(31) dos Santos RV, Titus RG, Lima HC. Objective evaluation of skin prick test reactions using digital photography. Skin Res Technol 2007; 13(2):148-53.

(32) Haahtela TM. The prevalence of allergic conditions and immediate skin test reactions among Finnish adolescents. Clin Allergy 1979; 9(1):53-60.

(33) Turkeltaub PC, Rastogi SC, Baer H, Anderson MC, Norman PS. A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. J Allergy Clin Immunol 1982; 70(5):343-52.

(34) Turkeltaub PC. Biological standardization based on quantitative skin testing--the ID50 EAL method (intradermal dilution for 50 mm sum of erythema diameters determines the allergy unit). Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1987;(80):169-73.

(35) Dreborg S, Basomba A, Lofkvist T, Moller C, Holgersson M. Suppression of immediate skin reactions by immunotherapy with birch, D. farinae and wall pellitory allergen preparations. Evaluation of different skin prick test methods. The skin prick test. Metodological studies and clinical applications. Linkoping: Linkoping University, 1987: 133-48.

(36) Dreborg S, Nilsson G, Zetterstrom O. The precision of intracutaneous skin test with timothy pollen allergen preparation using two different techniques. Ann Allergy 1987; 58(1):33-5.

(37) Allergy Diagnostic Testing: An Updated Practice Parameter, AAAAI & ACAAI.. AAACI. 2008; 100, 3, Supplement 31 – 149.

(38) Niemeijer NR, Goedewaagen B, Kauffman HF, de Monchy JG. Optimization of skin testing. I. Choosing allergen concentrations and cutoff values by factorial design. Allergy 1993; 48(7):491-7.

(39) Dreborg S, Rimas M, Bjorksten B, Kjellman M. Diagnosis of rhino-conjunctivitis (RC) by history (CH), skin prick test (SPT) and conjunctival provocation tests. Discussion on definition of disease. J Allergy Clin Immunol 83[2], 265. 1989.

(40) Dreborg S. Diagnosis *in vivo* and *in vitro*. In: Mygind N, Nacleiro R, editors. Allergic and non-allergic Rhinitis. Clinical aspects. Copenhagen: Munksgaard, 1992: 82-4.

(41) Dreborg S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. Allergy 1993; 48(7):473-5.

(42) Dreborg S. The optimum concentration for skin prick testing. Allergy 1997; 52(11):1142-3.

(43) Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA. Survey of fatalities from skin testing and immunotherapy 1985-1989. J Allergy Clin Immunol 1993; 92(1 Pt 1):6-15.

(44) Bukantz SC, Lockey RF. Adverse effects and fatalities associated with subcutaneous allergen immunotherapy. Clin Allergy Immunol 2004; 18:711-27.

(45) Haahtela T, Jokela H. Influence of the pollen season on immediate skin test reactivity to common allergens. Allergy 1980; 35(1):15-21.

(46) Moller C, Elsayed S. Seasonal variation of the conjunctival provocation test, total and specific IgE in children with birch pollen allergy. Int Arch Allergy Appl Immunol 1990; 92(3):306-8.

(47) Taudorf E, Malling HJ, Laursen LC, Lanner A, Weeke B. Reproducibility of histamine skin prick test. Inter- and intravariation using histamine dihydrochloride 1, 5, and 10 mg/ml. Allergy 1985; 40(5):344-9.

(48) Moller C, Bjorksten B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. Allergy 1984; 39(1):37-41.

(49) Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. Allergy 2006; 61(6):671-80.

(50) Pierson-Mullany LK, Jackola DR, Blumenthal MN, Rosenberg A. Evidence of an affinity threshold for IgE-allergen binding in the percutaneous skin test reaction. Clin Exp Allergy 2002; 32(1):107-16.

(51) Strachan D, Sibbald B, Weiland S, it-Khaled N, Anabwani G, Anderson HR et al. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC). Pediatr Allergy Immunol 1997; 8(4):161-76.