Evaluation of Skin Reactivity: The concept of Histamine Equivalent Allergen Threshold Concentration (C\text{ha})

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Abstract

Background: Skin prick testing is the most common diagnostic tool used by allergists. There are limited, international rules on interpreting and reporting skin test results in a meaningful way.

Aim: This communication describes methods to express the results of skin prick tests in a meaningful way. It is recommended to use allergen extracts with defined composition, potency and stability, to keep the precision of SPT within acceptable limits by using duplicate tests, and to regularly calculate the c.v. If duplicate tests cannot be performed for practical and or psychological reasons, e.g. in small children, then it are proposed that regular proficiency tests are performed and reported. The method of estimating the allergen threshold concentration, histamine equivalent allergen concentration C\text{ha}, is described

Conclusion: By adjusting the allergen wheal response to that of histamine, C\text{ha}, differences in techniques between personnel and centres can be minimized and changes in skin reactivity can be calculated as a threshold concentration. To document the skin reactivity and changes over time it is even proposed to report the mean histamine wheal response of groups and of testing personnel at all-time points of therapeutic trials and in practice over time.

Keywords: Skin prick test; Allergen; Histamine; Histamine equivalent concentration; Threshold concentration; Proficiency testing; Cut-off; Precision; Technique

Abbreviations A: Area of the allergen or histamine wheal; AAAAI: American Academy of Allergy, Asthma and Immunology; ACAAI: American College of Allergy, Asthma and Immunology; C: Concentration; C\text{ha}: Histamine equivalent allergen concentration; CPT: Conjunctival Provocation Test; c.v.: Coefficient of Variation; D: Diameter; DBPCFC: Double-blind Placebo Controlled Food Challenge; EAACI: European Academy of Allergy and Clinical Immunology; SD: Standard Deviation; SPT: Skin Prick/Puncture Test; s-IgE: Allergen IgE Antibodies in Serum

Introduction

The skin prick/puncture test (SPT) is the most common diagnostic tool within allergology. There are many examples of practice parameters or position papers such as “Allergy diagnostic testing: an updated practice parameter” [1], the “EAACI position paper on Allergen standardization and skin tests” [2], “The skin prick test-European standards” [3], and the “Global Atlas of Allergy” [4]. However, despite these statements, most publications using SPT for diagnosis and/or evaluation of changes in skin sensitization do not contain relevant information on the test procedure and does not use meaningful methods for evaluation of results. It is therefore necessary to discuss how to evaluate those results scientifically and clinically.
\[
\frac{(d_1+d_2)}{2}=D
\]

or Calculate the area by planimetry or digitizer. Poulsen et al. developed a simple scanning program to estimate the wheal area [8,9]. This program is no longer in use but there are modern programs for estimation of cell area, e.g. cell sens software [10].

The wheal should be surrounded by a flare [1].

The results should be registered on a record sheet (and in the computer program). Drawings and measurements should be preserved for possible follow up and control.

One report suggests the longest diameter correlates better than the mean diameter with the area of the wheal [11]. However, this correlation does not prove the longest diameter is a better measure than the mean diameter as a measure of skin sensitivity. The question is if there is a better correlation between the patient’s shock organ sensitivity and different expressions of skin sensitivity. Such data are not available for the longest diameter. However, changes in conjunctival sensitivity correlate well with changes of wheal area and mean wheal diameter [12].

There are other methods that have been used for scientific investigations, for example

- estimating the blood flow within and around the wheal area [13],
- estimating electrical impedance [14],
- using thermography [15],
- using digital photography [16],
- and carrying out 3-D scanning [17].

Evaluation of skin response

Expression of test results in principle, there are five possibilities:

- Using the mean (D) of the longest (d_1) and the midpoint orthogonal (d_2) diameters,
- Using the area.
- Estimating the allergen mean wheal diameter (wheal area) in relation to that of histamine, using a +++ system [18]. The first three approaches are well known. However, they do not express changes in relevant terms [7]. The third method relates allergen wheal response to that of histamine. However, it is a non-precise, semi-quantitative method not reporting allergen threshold concentrations [18].
- Using the method proposed by Durham [19] of using the allergen concentration eliciting a wheal with 6 mm diameter as a threshold concentration. The forth method does not correct for differences in SPT technique [6] or for changes in histamine sensitivity due to changes in allergen sensitivity over time or due to therapy [12].
- Calculating the allergen wheal size in percent of the histamine wheal size.

When using the fifth method allergen sensitivity is related to the histamine reactivity, but it does not give any information on the sensitivity as expressed in allergen concentration and such data cannot be used to determine the degree of change in allergen threshold concentration during e.g. immunotherapy. The changes in a double blind placebo controlled study are shown in Figure 1a.

Figure 1 (a,b): The change in skin reactivity during immunotherapy of two groups (mean) with a D farinae extract (major allergen content = 100 μg/ml ± a factor 2 (44)) and placebo over three years, expressed as the allergen skin group mean wheal response in percent of the individual histamine wheal. The placebo group received active treatment after one year. Changes in skin sensitivity from before treatment to after immunotherapy (permission by the main investigator). b. The same data recalculated using the Cha D farinae and placebo before and after one, and one and a half years of immunotherapy, expressed as Cha, using the slope 0.2 for diameter (Cha=[Dh/Da]* conc. allergen used). After one year, the placebo group received active treatment. Changes in relation to skin sensitivity before immunotherapy. The estimated change in skin sensitivity between the active and placebo treated groups after 12 months was about 70-fold. The change in skin sensitivity from before therapy to after 18 (initially active group) and six months (placebo group receiving therapy between 12 and 18 months) of immunotherapy was more than 100-fold (permission by the main investigator).
Calculating the histamine equivalent threshold concentration, \( C_{ha} \)

Finally, the sixth method has the advantage of expressing the result in relation to histamine (6) and is also related to the skin sensitivity (histamine), expressing the allergen sensitivity as the histamine equivalent allergen concentration, \( C_{ha} \) [7].

The same data as in Figure 1a are shown in Figure 1b, showing the \( C_{ha} \) before and after 12 and 18 months of immunotherapy. It demonstrates the about 100-fold difference in change in skin sensitivity between active and placebo treatment from before to after 12 months of immunotherapy and the more than 100-fold reduced skin sensitivity in both groups after 18 and 6 months of immunotherapy, respectively.

The mean allergen wheal diameter or the allergen wheal area is used in routine and in most published trials. This is simple, but does not give any information about the sensitivity of the patient that is comparable to in vivo threshold concentrations (PC20, PD20, CPT/NPT threshold concentrations or the double blind, placebo-controlled food allergen challenge, DBPCFC, threshold concentrations) and in vitro tests with a documented cutoff. Furthermore, it does not enable calculation of changes in skin sensitivity to allergens, with or without therapy, as expressed in changed threshold concentration, over time or between groups in cross-sectional studies.

After the introduction of SPT, European manufacturers started delivering extracts in one concentration and, even in the US it became common to use one dilution of the stock solution for SPT. This method does not deliver an allergen concentration but delivers a diameter (or area).

Prior to the widespread use of SPT, intra-dermal skin testing was used for end-point titration. Then the endpoint of allergen, was used as a measure of skin sensitivity and was often used to determine the starting dose for immunotherapy. The end point concentration as determined by SPT correlates with the threshold concentration using the gold standard for estimation of the histamine equivalent allergen concentration, Cha, the parallel line bio-assay [20,21], and with shock organ sensitivity as expressed by CPT [12,22].

Determining the concentration of allergen eliciting a wheal of the same size as that of histamine reduces the difference between testing personnel, centers and test occasions [6]. Using the slope (b) of the allergen dose response relationship [23], the allergen response can be expressed as a threshold concentration (Cha) [7], as illustrated in Figure 2a. The mean slope (b) of the allergen dose-response relationship (Log D (mean wheal diameter)=a (intercept with the Y-axis)+b (the slope) log (concentration of allergen)) was independently calculated by Dreborg et al. [23-26], and Björkstén et al. [27] (non-published, observation data mentioned in [27]) and was found to be 0.2. The group of Dreborg used parallel line bio-assay [21,22] in several publications for determination of the biological activity of allergen extracts. That material delivered information on the median slope (b) of the allergen dose response relationship. Initial preliminary studies were carried out using histamine dihydrochloride 1 mg/ml [24-26] and then a final study used histamine dihydrochloride 10 mg/ml as standard [23]. In total more than 700 adults (15-50 years) were tested with in-house reference standards (IHR) of two grass species, birch, alder and hazel, mugwort, two species of Parietaria, two other weeds (English Plantain and Goose-foot), two pets (cat and dog), two molds (Alternaria alternata and Cladosporium herbarum), two mites (Dermatophagoides pteronyssinus and D. farinae). Each species was tested in at least 20 patients. The pet, grass, tree and mite extracts were tested in several European regions with similar results, i.e. overlapping c.i.. Björkstén et al. [27] tested 708 adolescents (aged 15-17 years) and 220 adults with 43 allergen extracts from different manufacturers to determine the potency of the extracts in HEP and the differences between suppliers.

The histamine equivalent allergen concentration (Cha) can be calculated based on the formula [2] derived from the allergen dose response relationship (Log D=a+b\( \log C \)) [7]:

\[
C_{ha} = \frac{D_h}{D_a} \times \text{conc. allergen used, (2)}
\]
The concept of Histamine Equivalent Allergen Threshold Concentration ($C_{ha}$). J Med Diagn Meth 6: 242. doi:10.4172/2168-9784.1000242

$C_{ha}=([Dh/Da]^{0.2} \times \text{conc. allergen used or } C_{ha}=([Dh/Da]^{3} \times \text{conc. allergen used})^{[7,27]}$. Formula (3) can then be used to determine the difference in skin sensitivity between two time points or trials.

$C_{ha}$ time one and $C_{ha}$ time two [12]. (3)

This describes the differences in concentration, e.g. from before to after therapy or between samples of patients. However, the difference can also be expressed as a ratio providing the fold-increase or decrease in skin sensitivity [12].

$C_{ha}$ time two/$C_{ha}$ time one (4)

These formulas are easy to introduce in an Excel spreadsheet. The $C_{ha}$ is much more useful than the wheal diameter or area and can be used for estimation of changes in skin sensitivity during therapy, e.g. with antihistamines or by immunotherapy [12]. However, when using the results of skin prick tests for this purpose, the technique must be optimal with a c.v. less than 20% (or optimally <10%) using the wheal diameter. Small changes in wheal mean diameter, D, mean large changes in skin sensitivity. A high sensitivity is expressed by lower $C_{ha}$ values, i.e. larger wheals indicate lower $C_{ha}$ and smaller allergen wheals correspond to higher $C_{ha}$, Table 1a.

Table 1b illustrates the relation between changes in allergen wheal diameter, D, and the fold change in skin sensitivity to the tested allergen. It should be noted that small changes in D at low response levels correspond to major changes in skin sensitivity. Changes including wheals less than 3 mm in D are presented in brackets due to the currently generally accepted cut-off at 3 mm in D. However, 2 mm in D is included to illustrate that a change from 3 to 2 mm in D corresponds to a 7.6-fold change in skin sensitivity, i.e. to about 13% of the pretreatment/earlier skin sensitivity. That should be compared to the decrease in symptom scores during antihistamine or cortisone therapy, mostly to about 70% of pretreatment symptoms, i.e. less than 2-fold.

[Table 1b]

<table>
<thead>
<tr>
<th>Difference mm</th>
<th>Fold-change</th>
<th>Difference mm</th>
<th>Fold-change</th>
<th>Difference mm</th>
<th>Fold-change</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-12</td>
<td>3.1</td>
<td>10-7.5</td>
<td>4.2</td>
<td>5-4.25</td>
<td>2.3</td>
</tr>
<tr>
<td>15-9</td>
<td>12.9</td>
<td>10-6.5</td>
<td>8.6</td>
<td>5-3</td>
<td>12.9</td>
</tr>
<tr>
<td>15-7.5</td>
<td>32.0</td>
<td>10-5</td>
<td>32.0</td>
<td>(5-2)</td>
<td>(97.7)</td>
</tr>
<tr>
<td>15-6</td>
<td>97.7</td>
<td>10-4.25</td>
<td>72.1</td>
<td>4.25-3</td>
<td>5.7</td>
</tr>
<tr>
<td>12-10</td>
<td>8.6</td>
<td>6.5-5</td>
<td>3.7</td>
<td>(4.25-2)</td>
<td>(43.3)</td>
</tr>
<tr>
<td>12-7.5</td>
<td>10.5</td>
<td>6.5-4.2</td>
<td>8.4</td>
<td>(3-2)</td>
<td>(7.6)</td>
</tr>
<tr>
<td>12-6</td>
<td>32.0</td>
<td>6.5-3</td>
<td>47.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-5</td>
<td>79.6</td>
<td>(6.5-2)</td>
<td>(362.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1b: The table illustrates the relationship between changes in allergen wheal diameter, D, and the fold-value of the change in skin sensitivity to the tested allergen. It should be noted that small changes in D at low response levels correspond to major changes in skin sensitivity. Changes including wheals less than 3 mm in D are shown in brackets due to the current generally accepted cut-off at 3 mm. It should be noted that a change from 3 to 2 mm allergen wheal D corresponds to a change in skin sensitivity of around 7-fold, i.e. to about 13% of the pre-treatment sensitivity. In comparison, the decrease in symptoms during antihistamine or cortisone therapy is mostly found to be about 70%, i.e. less than a 2-fold change in symptom scores.

In Figure 1b the mean allergen wheal sizes in % of the mean histamine wheal diameter (Figure 1a) have been recalculated into $C_{ha}$ before and after one year of immunotherapy [12,28]. Another theoretical example of results using $C_{ha}$ before and after immunotherapy is shown in Table 2. One allergen is used for immunotherapy, the reactivity to the other is just observed, such as by Dreborg et al. [29]. It has been shown that the histamine reaction is reduced during immunotherapy [12,30]. The calculated change in sensitivity to allergens is influenced by the change in histamine reactivity as illustrated in the table. However, the difference in changes between active and placebo does not change. This is an important observation. The difference in changes in relation to non-treatment or placebo is the crucial parameter when evaluating any type of anti-allergic therapy.
### Table 2: One allergen is used for immunotherapy, the reactivity to the other is just observed. Three examples are shown in the table. The response to histamine is the same or is reduced by 1 and 2 mm diameter during immunotherapy. The calculated changes in sensitivity to the respective allergens are influenced by the change in histamine reactivity, as illustrated by the three cases. However, the differences in change are the same.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Immunotherapy</th>
<th>After Immunotherapy</th>
<th>Fold-change within group</th>
<th>Fold-difference in change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dh</td>
<td>Da</td>
<td>C&lt;sub&gt;ha&lt;/sub&gt;</td>
<td>Dh</td>
<td>Da</td>
</tr>
<tr>
<td>Histamine wheal unchanged during immunotherapy</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Active allergen</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Placebo allergen</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Histamine wheal reduced by 1 mm during immunotherapy</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Active allergen</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Placebo allergen</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

**Factors of importance for meaningful determination of C<sub>ha</sub>**

**Cutoff**

The question of when a test is positive or negative has been subject to lively discussion as long as skin testing has been part of allergy diagnosis. In studies, as well as in clinical practice, defining positive and negative results is important.

Actually, for decades there was no agreement on how to define a positive SPT. In 1987, Dreborg et al proposed ≥ 7 mm (≈ ≥ 3 mm D) to be the cut-off that was adopted by the Nordic Guidelines [31] and the EAACI position paper [2]. However, there was no documentation of that limit at that time. One factor influencing the proposal was the higher c.v. at low response levels [23,26]. There must be a clear definition of the background using the device [1], diluent and technique used in the office/study. The cut-off should be the upper limit of the background, i.e. the background mean +3.3 standard deviations (s.d.) [32]. The background is determined by testing a number of patients with a number of tests with the negative solution. Later, Nelson et al. [33,34] reported the background of a number of devices used in the US, using 80 tests with a negative control solution and calculating the background [33,34], and thereby the cut-off, in agreement with [32] (Table 3). For devices unique to Europe and other areas, data are missing. For those, the cut-offs should be defined.

<table>
<thead>
<tr>
<th>Device 1</th>
<th>0.99 Quintile of reactions at the negative control sites, mm</th>
<th>Device 2</th>
<th>0.99 Quintile of reactions at the negative control sites, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartest (HS) puncture</td>
<td>0</td>
<td>DuoTip (Lincoln) twist</td>
<td>3.5</td>
</tr>
<tr>
<td>Smallpox needle (HS) prick</td>
<td>0</td>
<td>Bifurcated needle (ALO) prick</td>
<td>4</td>
</tr>
<tr>
<td>DuoTop (Lincoln) prick</td>
<td>1.5</td>
<td>MultiTest (Lincoln) puncture</td>
<td>4</td>
</tr>
<tr>
<td>Lancet (HS)</td>
<td>2</td>
<td>Bifurcated needle (ALO) puncture</td>
<td>4.5</td>
</tr>
<tr>
<td>Lancet (ALK)*</td>
<td>3</td>
<td>Quick Test (Pantrex)</td>
<td>4</td>
</tr>
<tr>
<td>DermaPICK II</td>
<td>0</td>
<td>Greer Track (Greer)</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**HS, Hollister Steer; Greer, Greer Laboratories; ALO, Allergy Labs of Ohio; Lincoln, Lincoln Diagnostics; ALK, ALK.*Not the ALK lancet used in Europe.**

**Table 3:** The size of wheals that are larger than 99% of the wheals with saline, using the same device on the subject’s back by the same operator (n=80) [1]. These data are US data and are not applicable to European devices.
Precision

The reliability of the results of diagnostic tests is depending on the precision of the estimate. This is true for SPT and is of major importance when evaluating SPT results for screening, diagnosis as well as using C_{eq} as a measure of the response.

In vitro tests should always have a documented precision (c.v.). The c.v. of in vitro tests is most often low, less than 10%. There are few reports on the precision of SPT. Aas [35] reported the precision (c.v.) of SPT based on the mean wheal diameter, using the methods of Pepys [36,37], Brown [38] and a multi-test device. The c.v. varied from 8% using the Pepys’ method [36], with a short beveled needle, to 30% with a multi-test device using the mean wheal D (Table 4). In major European centers [23], the c.v. varied from 15% up to 145% for allergens and from 12% to 65% for histamine, as calculated on wheal areas using the Osterballe needle with 1 mm point and shoulders preventing further penetration [39]. The c.v. calculated on the diameter is half that of the c.v. calculated on the area (area of a circle πr², the index 2 makes the difference). In that report, quadruplicate tests with each of three concentrations of allergen (about 9000 tests) and histamine dihydrochloride 1 and 10 mg/ml was used (about 5000 tests). However, duplicate tests are sufficient for calculation of the c.v. Examples are shown in Figure 3 (a,b).

Table 4: Tests performed according to Pepys using a short beveled needle, with the Morrow-Brown needle and with a multi-test device, testing the same 80 patients with histamine dihydrochloride 1 mg/ml, according to Aas. This assistant should obviously use the method of Pepys, causing the largest wheals, the lowest range of histamine weal sizes and the lowest c.v. D is the mean diameter, i.e. mean of the longest and the midpoint orthogonal diameters; s.d. is the standard deviation; c.v. is the coefficient of variation.

<table>
<thead>
<tr>
<th>Device</th>
<th>n</th>
<th>D</th>
<th>s.d.</th>
<th>c.v.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short beveled needle</td>
<td>80</td>
<td>6.1</td>
<td>0.51</td>
<td>8</td>
<td>5 - 7</td>
</tr>
<tr>
<td>Morrow Brown needle</td>
<td>80</td>
<td>5.7</td>
<td>0.55</td>
<td>10</td>
<td>4.5 - 7</td>
</tr>
<tr>
<td>Multi-test (brand not defined)</td>
<td>80</td>
<td>4.5</td>
<td>1.35</td>
<td>30</td>
<td>2 - 7</td>
</tr>
</tbody>
</table>

Documentation of the precision of the skin prick test method

In principle, there are (at least) two possibilities: To perform duplicate tests with histamine and all allergens, or to use proficiency testing [1,40] (and in the online repository). In small children single tests are acceptable, provided proficiency tests are performed at intervals.

Duplicate tests

In most cases, it is easy to perform duplicate tests instead of single tests. The extra time spent is limited. The extra material needed is negligible too, since the same device/needle/lancet can be used for the second test with the same allergen in the same patient. When performing Prick-Prick tests a new device should be used for each patient, allergen, but can be used for two pricks with the same allergen in the same patient.

Figure 3: Data on individual wheal diameters, the mean and median diameters and c.v. are presented in Table 6. Tests performed using histamine dihydrochloride 10 mg/ml in one person using the Osterballe lancet with one 1 mm tip and shoulders. Note the difference in size between the two testers. a. From a center using duplicate tests in practice (permission by the tester at EAACI 2014). b. From another center (permission by the tester at EAACI 2016).

The precision is given by the coefficient of variation, c.v., which is derived from the formula:

\[
s_d \times 100 = \text{c.v. percent (5)}
\]
Mean skin sensitivity using 3 mm as cut diameter. With narrow limits, the c.v. increases at lower response levels close to the cut value of SPT in clinical practice. Wheals in the same patients. It is proposed this size should be 7 mm in at wheals 7-10 mm.

Table 5: Prick tests performed on the volar aspect of the forearm by two testers using histamine dihydrochloride 10 mg/ml, Figure 3 (a,b).

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Mean</th>
<th>s.d.</th>
<th>c.v.</th>
<th>Range</th>
<th>Significance of difference between means p&lt;0.00001</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>9.0</td>
<td>6.2</td>
<td>7.6</td>
<td>6.0 - 7.0</td>
<td>Histamine conc. for the same result</td>
</tr>
<tr>
<td>171 mg/ml</td>
<td>5.2</td>
<td>4.2</td>
<td>4.7</td>
<td>6.5 - 8.25</td>
<td>6.0 - 4.0</td>
</tr>
</tbody>
</table>

Table 6: According to the AAAAI Practice Parameter 2008, Bernstein et al. [1].

Suggested Proficiency Testing and Quality Assurance

Technique For Prick/Puncture Skin Testing
- Using desired skin test drive, perform skin testing with positive (histamine 1-10) and negative controls (saline 1-10) in an alternate pattern on a subject’s back.
- Record histamine results at 8 minutes by outlining wheals with a felt tip pen and transferring results with transparent tape to a blank sheet of paper.
- Record saline results at 15 minutes by outlining wheal and flares with a felt tip pen and transferring tape to a blank sheet of paper.
- Calculate the mean diameter as (D+d)/2; D=largest diameter and d = orthogonal or perpendicular diameter at the largest width of D

Histamine
- Calculate the mean diameter of each wheal
- Calculate the s.d.
- Determine the coefficient of variation (c.v.) = s.d. /mean or the c.v. % = s.d. * 100/mean
- Quality standard should be c.v. less than 30% saline

Saline
- All negative controls should be ≤ 3 mm wheals and flares should be ≤ 10 mm in Diameter.

Furthermore, it must be considered that in most diagnostic systems, the c.v. increases at lower response levels close to the cut off concentration. In two reports [23,25], the c.v. was reported at different response levels. In both reports the c.v. of allergen SPT was about 50% at wheals 7-10 mm² (3-3.5 mm diameter), at higher levels about 30% with narrow confidence limits. Thus, the proposed simple control method using the ± 1 mm is not based on solid scientific data, but should be considered a minimum requirement in practice.

Proficiency tests
The first aim of proficiency testing [1,40] is to train assistants to achieve high precision, both for clinical trials and for improving the value of SPT in clinical practice. The second aim of proficiency testing is to train all assistants in an office or in offices participating in multicenter clinical trials to obtain the same size of the histamine wheals in the same patients. It is proposed this size should be 7 mm in diameter. The aim is to obtain comparable results from the different nurses or centers. It also makes it possible to note at about 70-fold decreased skin sensitivity using 3 mm as cut off and more than 500-fold decrease using 2 mm mean wheal diameter as cut off. The third aim of proficiency testing is to maintain high precision when using single tests, e.g. in children. Then, the testing personnel must document a consistent technique at intervals. Such a test is illustrated in Figure 3a and b and the results are shown in Table 5.

When ocularly comparing two tests, the rule is that at normal response levels, between approximately 4 and 8 mm D, a difference of ± 1 mm can be accepted. At the same time it should be remembered that the difference in strength of an extract causing a 4 mm wheel D to that causing a wheal with a D of 8 mm is around 32-fold [41]. Thus, if an extract that is labelled 10 U gives a 4 mm wheal D, then an extract labelled 320 U induces a wheel with about 8 mm D in the same patient. Similarly, a change in wheel D from 8 to 4 mm during therapy indicates 32-fold reduced skin sensitivity, i.e. less than 3% of the pre-treatment skin sensitivity, and the difference between 4 and 6 mm, i.e. 5 ± 1 mm wheal is 7.6-fold and 4 ±1 mm means a 13-fold difference in skin sensitivity.
The effect of pressure on the lancet/device

Clinically, it has been obvious that the higher the pressure on the lancet, multi-test etc., the larger the histamine or allergen wheal response. However, the first well designed study on the importance of differences in pressure on the device was published recently by Andersen et al. [42]. They used an equipment delivering an exact, predetermined pressure on the lancet, for the first time proving the association between pressure applied and the size of the skin wheal response. The effect of using different test techniques by different testing personnel and between centers participating in multicenter studies is illustrated in Table 5. Both testers used histamine dihydrochloride (histamine HCl, b=0.17 [23]), results were obtained by using equation (2) needs only the use of histamine 10 mg/ml and one allergen concentration both tested in at least duplicate (4 tests) using the simple equation (2) that can be inserted in e.g. an Excel sheet automatically delivering the result. The methodology is simple and should be obligate in scientific studies and even useful in clinical practice. The Cha delivers a threshold concentration making possible estimation of changes of skin sensitivity in terms of change in concentration similar to what is obtained by all other challenge tests.

Limitations and contributions of the Cha method for estimation of skin reactivity:

Contributions of estimation of Cha for estimation of skin reactivity:
In scientific work, the histamine equivalent allergen concentration, Cha can be calculated by parallel line bioassay that needs testing with at least three ten-fold concentrations of allergen and histamine HCl 10 mg/ml, all four in at least duplicate (8 prick tests) and complicated mathematical procedure. Calculation of Cha using equation (2) needs only the use of histamine 10 mg/ml and one allergen concentration both tested in at least duplicate (4 tests) using the simple equation (2) that can be inserted in e.g. an Excel sheet automatically delivering the result. The methodology is simple and should be obligate in scientific studies and even useful in clinical practice. The Cha delivers a threshold concentration making possible estimation of changes of skin sensitivity in terms of change in concentration similar to what is obtained by all other challenge tests.

Limitations
The only limitations are:

- the estimation of Cha necessitates training to obtain meaningful data
- the training has been questioned by some assistants and doctors since it is perceived as a threat against their professional skill.

Conclusions and suggested recommendations
Based on previous documentation it is recommended:

- Using well standardized extracts/components, with known total allergenic potency with known stability.
- Using a negative control solution for documentation of each person's background.
- Using a positive control for documentation of skin reactivity, technician's skill and for evaluation of skin reactivity.
- Registering and store the wheal (and erythema) size.
- Determining the mean wheal (and erythema) diameters or areas.
- Aiming at 7 mm mean histamine diameter, using 10 mg/ml in defined patients.
- Documenting the precision by duplicate tests and calculated c.v., or if this is not possible, the c.v. obtained by proficiency tests performed should be reported.
Recent advances described and recommended are summarized below

Methods for estimation of the histamine equivalent allergen concentration, $\mathrm{C}_{\text{eq}}$, i.e. the proposed SPT threshold concentration.

Methods for estimation of changes of $\mathrm{C}_{\text{eq}}$ during therapy and over time, allowing for estimation of change in threshold concentration and or fold-change in threshold concentration.

Relevant parts of these recommendations should be applied also to clinical practice.

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References
