## Appendix 2

**Proposed method for the evaluation of skin prick test data from clinical trials**

##### Test procedure

* Use a well documented device and methodology
* Use a defined CA. Preferably with known major allergen content by μg/ml or mmol/L, using an internationally acknowledged method
* Train the personnel to perform proficiency tests using 20 histamine SPT on the forearms of 3-4 people until the precision is good, i.e. the c.v. not higher than 20 % (diameter), to document the precision([1](#_ENREF_1)).
* Use at least three ten-fold CA, as a hallmark about 1, 10, 100 µg of major A([2](#_ENREF_2), [3](#_ENREF_3)). Ten micrograms corresponds the median CH10 in European patients, and two concentrations, one 10 times stronger and one ten times weaker ([2](#_ENREF_2), [4](#_ENREF_4)).
* Calculate the mean (or if more than duplicate tests, geometric mean) DH10 or AH10 and DA or AA, respectively, per concentration and patient
* The mean (or geometric mean) is used for calculations
* Use of DA or AA of the allergen wheal most similar to the DH or AH for thecalculations
* The parallel tests should be similar, i.e. with a c.v. less than 20 % computed on the DA or 40 % computed on the basis of AA. During the trial it is possible to check that D with the same material and concentration do not differ more than ± 1 mm in D
* Repeat and document the result of the proficiency tests every month during the trial to document the c.v. and the possible shift in histamine mean wheal size during the trial([1](#_ENREF_1)).

The CA eliciting a wheal of the same size as that of histamine HCl 10 mg/ml is calculated, using the size of the allergen wheal most similar to the histamine reaction, assuming the same b for A and H (bA ≈ bH).

The threshold CA = []5 \* CA used. Use Excel or similar for calculations as given below:

**Example: Calculation of the allergen concentration eliciting a wheal of the same size as that of histamine HCl 10 mg/ml, CH10. The allergen potency 10 Units is arbitrary** (should be changed to the unit labelled, preferably µg major allergen or BU, ABU, HEP, IR , etc.). Active Excel file (link to be inserted).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **1** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** |
| 2 | **Pat.** | **Allergen** | **1st test occasion** | | |  | **2nd test occasion** | | | **Times change** |
| **3** | **DH** | **DA** | **A x unit** |  | **DH** | **DA** | **A x unit** |
| 4 | 1 | Birch | 5 | 8 | 0,95 |  | 6 | 4 | 75,94 | 79,63 |
| 5 | 1 | Timothy | 5 | 4 | 30,52 |  | 6 | 4 | 75,94 | 2,49 |

The formula inserted in cells E4 and I 4 “= Index(C4/D4;5)\*10” and in E 5 and I 5: “= Index(C5/D5;5)\*10”, resp.

See Excel file.

#### Abbreviations

|  |  |
| --- | --- |
| A | Area |
| AA | Mean wheal area obtained with the same allergen concentration in the same patient |
| AH | Mean wheal area obtained with histamine reference in the same patient |
| AH10 | Mean wheal area obtained with histamine 10 mg/ml in the same patient |
| D | Diameter |
| DA | Mean wheal diameter obtained with the same allergen concentration in the same patient |
| DH | Mean wheal diameter obtained with histamine reference in the same patient |
| DH10 | Mean wheal diameter obtained with histamine 1 mg/ml in the same patient |
| CA | Concentration of allergen |
| CH | Concentration of histamine |
| drr | Dose response relationship |
| bA / b H | The slope of the allergen / histamine drr |
| a | The intercept of the drr |

#### Legends to figure

Proposed method according to ***Appendix 2,* Clinical trials**

Duplicate tests (at least) are performed with at least three ten-fold concentrations, as a hallmark 1, 10, 100 µg of major allergen. The CA eliciting a wheal response closest in size to DH10 or AH10  should be calculated according to the formula:

= []5\*CA used, or with A = []2.5\*CA used.in the investigation.

1. Proficiency testing: Skin prick test [Internet]. 2013 [cited 2013-02-22]. Available from: https://aaaai.confex.com/aaaai/2013/webprogramhandouts/Session1315.html.

2. 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.

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

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